MOSQUITO STUDIES (Diptera, Culicidae)

II. METHODS FOR THE COLLECTION, REARING AND PRESERVATION OF MOSQUITOES[1]

By

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INTRODUCTION

The primary purpose of this paper is to provide uniform methods for the collection, preservation and rearing of material for the project "Mosquitoes of Middle America" (Belkin et al. 1965) but the methods discussed are general enough to be applicable in similar studies in other areas. No attempt is made to present an exhaustive review and discussion of the multitude of techniques which have been used successfully by hosts of investigators for various specific problems. The emphasis here is on methods suited for obtaining mosquito material for taxonomic studies of a general survey nature. Essential for this type of study is a large amount of uniformly prepared material with all the stages individually associated, general information on bionomics and conspicuous environmental factors and a sample from as many habitats as possible over a wide geographic range. Important considerations in selecting the methods and techniques adopted here have been simplicity and suitability for use under field and laboratory conditions in the tropics and standardization and simplification of records and labeling to minimize errors and to save time. A great deal of effort and experimentation has been put into developing these techniques and record forms. A variety of equipment, forms and methods have been tested for several years in Middle America, the United States, the South Pacific islands and New Zealand before arriving at the recommendations presented here. A special effort was made to use light weight and compact equipment and supplies suitable for transportation by air and at the same time rugged enough to take abuse. We beg the indulgence of experienced laboratory and field investigators for presenting and describing in detail many elementary procedures but we have found through experience that most of these are not understood or followed even by trained technicians with the result that much valuable material is lost.

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COLLECTION RECORDS

COLLECTION FORM. A standard form (fig. 1) for recording all the data pertaining to a collection has been developed for field work in Middle America but it can be used anywhere in the world. The front of the card is primarily for recording information on the collection itself, the back for recording the individual rearings.

The form must be filled out in the field as completely as possible and the remainder added in the laboratory. A soft pencil (no. 2, 2.5 or equivalent) should be used for all entries. All measurements should be indicated in the metric system; if miles, feet or inches are used, the metric abbreviation (m) should be crossed out and the appropriate nonmetric unit clearly noted. There is a minimum of writing to be done (only in lined open spaces) on the form, the rest is to be done by circling or underscoring appropriate words or statements or by placing check marks (✓) or other signs in appropriate columns.

This collection form is intended to serve as a guide to the selection of places and methods of collecting adults and immatures in the field and therefore it is discussed in considerable detail below and in the following two chapters.

COLLECTION CODE AND NUMBER (LOT). Collections are identified (1) first by a code of 2 or more capital letters designating the country, collecting team or institution (2 or more codes are employed for one country when 2 or more teams are operating simultaneously from different bases) and (2) second by a collection or lot number. The collection numbers run in a regular sequence starting with 1 for every code. The collection number is used to identify all the material derived from that collection. It is usually subdivided into sublots and individual numbers to identify groups or individuals within a given collection (see SUBLOTS and INDIVIDUAL REARINGS). These subdivision numbers are separated from the collection number by a dash or hyphen (-) to the right of the collection number.

The standard collection form is used for a collection of immature stages or for a collection of adults; the same collection number should never be given to a mixed collection of adults and immature stages. However, a note should be made under REMARKS indicating that the two collections were made in the same locality. The single numerical sequence of lot numbers under the particular code is still followed irrespective of the type of collection, immature or adult.

In the case of immature stages, a separate collection number is assigned to every collection made from one individual breeding site, i.e. one ground pool, one location in a swamp, one crabhole, one treehole, one bromeliad, one pitcher, etc. Only rarely when the same and only one species is found in 2 or more identical breeding sites should a composite collection be made; a note should be made indicating this under REMARKS. In case of leaf axils or inflorescences from a single plant, collections from individual inflorescences or axils should be numbered separately if different species are suspected and a notation made of the habitat differences.

In the case of adults, a separate collection number is assigned to every collection in one specific site and for each specific type, i.e. biting and landing at one site in a wooded area at a particular time of day, or from one trap, from one crabhole, from one culvert, etc. Any composite collection should be explained under REMARKS. A collection of adults for progeny rearings must be assigned a number distinct from any general adult collection made at the same time and place.

LOCALITY AND RELATED INFORMATION. For the locality the specific geographical location of the area where a collection is made must be indicated so that the place can be readily found in the future. Reference may be made to kilometer posts or distance from a settlement on a specified highway. Be as specific as possible and if topographic or road maps are available indicate by the collection number the locality as accurately as possible on the map. As a rule, several collections will be made in one locality and it may be necessary to specify in more detail the exact location of each. The nearest town or settlement that can be located on a map must be given and, as indicated above, the distance (km) and direction (azimuth) of the locality in respect to the town should be given. The district (county, parish, etc.) and the province (state or other major division) and country should be given. The date and the name(s) of the collector(s) must be written in for every collection. If the elevation can be obtained from an altimeter or a map it should be given. Whenever possible a photograph should be taken of the general locality and environment and of the specific habitat, particularly in case of water-holding plants. The photographs should be identified by the collection number written with grease pencil on a

card or large cardboard or other object and placed in an appropriate place in the scene to be photographed. One photograph of a general locality may serve for many collections. Indicate by a check mark in the collection form that a photograph has been taken.

GENERAL ENVIRONMENT. For every collection, whether of immature stages or adults, all the items in this section must be considered very carefully and all the applicable ones circled or underscored in each of the statements 1 through 7. In addition, the information for statements 8, 9 and 10 should be filled in whenever the data are available. Any other pertinent information should be given in the REMARKS section.

1. Woody plants. Included in this category are trees, shrubs, brush, woody lianas and arborescent forms such as bamboo; woody epiphytes are excluded (see 3). The height scale is from 0 to 30 meters; indicate the height of the woody plants by encircling the proper numerals to show the range. In the density scale: 0 represents total absence of woody plants; 1, low density as exemplified by trees in a park; 2, medium density as in open woodland where one can walk through readily; 3, high density as in a dense forest impenetrable without cutting or moving vegetation out of the way; indicate the density by encircling the appropriate numeral(s), taking into account intermediate conditions and range of density.

2. Herbs and grasses. Included in this category are all non-woody plants other than epiphytes (see 3), even such forms as banana "trees," heliconias and terrestrial bromeliads; arborescent forms such as bamboo are excluded (see 1). The height scale in meters and the density scale should be filled out in the same manner and using the same general criteria as for woody plants.

3. Epiphytes. An important element in the environment of mosquitoes in the tropics are certain epiphytes which by virtue of the plant structure are capable of holding water often in considerable quantity; many species of bromeliads fall in this category. In the density scale: 0 represents total absence; 1, low density, a few plants per tree; 2, medium density, numerous plants; and 3, high density, covering nearly all available space.

4. Edge effect. Some of the most productive environments for mosquitoes are to be found along the edges of vegetation formations or artificial interruptions such as paths, roads, dikes, canals. The edge of a vegetation formation can be identified by the presence of many different types and species of plants and may extend for several meters inward from the actual margin.

5. Water effect. The bulk of mosquito breeding will be found in the areas surrounding permanent or temporary bodies or courses of water. Shore is used here in the broad sense to include the belt of vegetation surrounding the body or course of water such as the riparian environment around a stream bed, draw or canyon bottom.

6. Human effect. Contrary to general belief, undisturbed environments are not very productive except along edges. Human disturbance is a very important factor in increasing the edge effect. Virgin vegetation is considered here to be that which is not markedly affected by man or domestic animals. Clearing refers to partially cleared forest and young second growth following clearing. Grazing refers to disturbance due to domestic animals. A plantation is a partially or completely cleared area planted with woody crops. Cultivation refers to cultivated fields or partially cleared areas planted with herbaceous or grassy crops. A domestic environment is a rural or urban area immediately surrounding human dwellings and domestic animal quarters.

7. Light. The amount of light or shade in the general environment of the collecting site is an important factor and should be recorded in the general terms indicated.

8. Annual rainfall. Indicate the approximate mean annual rainfall in centimeters if reliable data are available. Give the data from the closest station if not available from the locality itself; under REMARKS indicate the station.

9. Rainy season. Circle or underscore the months during which the bulk of the precipitation falls.

10. Vegetation type. The specific type of vegetation in the area should be given. For the area covered in the project on the "Mosquitoes of Middle America," it would be primarily one of the following: tropical rain forest, subtropical rain forest, tropical deciduous forest, tropical montane forest (cloud forest), paramo, thorn forest, desert scrub, grassland, savanna, chaparral, pinyon-juniper woodland, pine barrens, temperate deciduous forest, montane coniferous forest; littoral (mangrove, saltmarsh). Alternately the system of Holdrige (1947) may be used.

IMMATURE STAGES. This section is further discussed and explained in detail in the chapter on COLLECTION OF IMMATURE STAGES. For each collection, only one breeding site is checked by circling the number in the list and underscoring, circling or filling in the appropriate description of the site and for the conditions of the breeding site, i.e. height of the site above ground, water, vegetation in breeding site and bottom.

ADULTS. This section is further discussed and explained in detail in the chapter on COLLECTION OF ADULTS. For each collection the exact site of collection is specified in detail and all the data are filled in, circled or underscored for type of collection (specify the type of light or other trap used), height above ground, host or bait and time of capture.

REMARKS. Special notes, explanations or clarifications of data given elsewhere on the form are recorded here.

SUBLOTS. This section is used to record the subdivisions of the material obtained in one collection. In the case of a collection of immature stages the sublot numbers (-1 to -9) are used to indicate the different species recognized in the collection and to record for these species the material that has been preserved directly from the field collection or from mass rearings. Similarly, the sublots should be used to identify the different species recognized in adult collections. Sublots are also used to identify progeny that have been reared from collected adults, a sublot being assigned to each individual parent female with her mass-reared progeny. The female parent should be identified by a capital P after the sublot number (e.g. -1P).

For each sublot write in the provisional identification (even if only to genus) and indicate by check mark in the appropriate squares the preservation of L (whole larvae), l (larval skins), P (whole pupae), p (pupal skins), M (males), F (females), E (eggs). The material belonging to a given sublot is labeled with the collection number (lot) followed by a dash or hyphen (-) and then the sublot number, e.g. 50-1 and 50-2 for sublots -1 and -2 in collection 50.

Provision is made in the form for a total of 9 species (or 9 progenies) in one collection. If more than 9 species (or parent females) are obtained in one breeding site (or adult collection) then a second collection number (lot), preferably in sequence, with identical data and with cross reference is assigned to allow the recording of the additional species (or progenies).

INDIVIDUAL REARINGS. The back of the card is used only to record individual rearings from field-collected immature stages or from progeny

rearings. The numbers are used to indicate individual specimens, the larval skin (l), the pupal skin (p) and the corresponding adult male (M) or female (F). Indicate by a check mark the stage preserved for each individual. Any stage that is lost is marked by a zero (0); a dead larva or pupa or a drowned adult preserved in alcohol is marked by a cross (+). For a drowned, partially emerged adult, place a check mark under "p" and a cross under "M" or "F." The species (or generic) name need not be written in at this time.

The three digit series (-100 to -114) is used for individual rearings made from wild collected pupae which it is not practical to sort to species. The two digit series are normally used for individual rearings from fourth instar larvae (field-collected or from progeny mass rearings). The series -10 to -19 for species or parent female of sublot -1; series -20 to -29 for species or parent female of sublot -2; series -30 to -39 for species or parent female of sublot -3; and so on to series -90 to -98.

In case more than 10 individual rearings are desired for one or more species, then one or more of the unused series may be employed for these, with an appropriate notation in the species column that this is a continuation of a particular series. The same applies when it is desired to rear individually more than 15 field-collected pupae; any of the unused larval series may be used, preferably the 90, 80, 70 and so on.

LABELING. All the material is identified only by numbers which refer always to the collection number (lot) entered on the collection form and its sublot and individual subdivisions as applicable. The collection form contains all the data pertaining to the collection and has a record of all the numbers. In the field there is no need to write in on the labels the country or team code letters; these should be clearly indicated later however before every shipment. The code will be shown on the permanent printed labels which will be attached to all the material at the laboratory where the material is processed and mounted, at UCLA for the project "Mosquitoes of Middle America" (Belkin et al 1965, fig. 2).

All labels should be on durable bond paper (20 lb) and written with a soft pencil (no. 2 or 2.5 or equivalent), never with ink.

For general collections (collections that are not subdivided into sublots) write a label showing only the collection number (lot); prefix the number with # or underscore it to eliminate confusion in numbers which can be read from either end.

For material which has been subdivided into sublots, write a label showing the collection number (lot) followed by a dash and then the sublot number, e.g. 50-1, 50-2 (see section on SUBLOTS above). Each vial and pillbox must have a label placed inside; never attach labels to the outside of containers.

For individual rearings, in order to save time, insure uniformity and minimize mechanical errors, use the printed individual labels provided for the purpose. These labels are printed in columns on a sheet. Cut the sheet into strips and keep strips of the same numbers in envelopes. The numbers correspond to those printed on the back of the collection form to identify individual rearings. In the 100 series the numbers on the strips are in duplicate (one for the pupal skin and one for the corresponding adult), in the 10, 20, 30 and so on to 90 series the numbers are in triplicate (one for the larval skin, one for the pupal skin and one for the corresponding adult).

To mark individual rearings in a given collection: (1) prepare a full strip of the proper series by writing in the collection number (lot) in front of the

dash or hyphen (-) preceding every individual number, (2) cut off a replicate set of an individual number starting at the top of the strip as needed, (3) attach the replicate set to the individual rearing container anchoring it under the lid, (4) cut off an individual label from the replicate set to mark the skin(s) and the adult as they are put in vials(s) or capsule; throughout the rearing there is always an individual label in a replicate set accompanying every stage to its ultimate preservation, (5) keep the strip of unused replicate numbers with the corresponding collection form or mass-rearing sublot until all isolations are completed then destroy the remaining part, (6) indicate by a check mark under the appropriate number on the back of the collection forms all the individual rearing numbers as they are being assigned.

FIELD BOOK. A small pocket notebook should always be carried in the field and used as a diary to record all incidental and detailed information for which space is not provided on the collection form. All the data on a particular collection should be entered under the collection number. An engineer's field book is particularly useful for this purpose because carbons of the notes are made at the time of the original entries and such copies can then be attached to the collection forms without recopying as would be necessary with an ordinary notebook. When completed a field book should be preserved as a permanent record of the field work.

COLLECTION OF ADULTS

EQUIPMENT. The basic equipment and supplies needed for the collection of adults are: (1) aspirators, (2) aerial nets, (3) oviposition vials, (4) killing tubes and jars, (5) plastic vials and plastic cups with caps and tissue paper, (6) pillboxes, (7) flashlight (torch) and (8) a collecting bag fully equiped as indicated in the GLOSSARY. Whenever possible, traps of various kinds, light traps, animal-baited traps and space traps, should be used (see below).

CAPTURING. Mosquitoes may be captured while in flight by means of a net with a fine nylon aerial bag. The apex of the bag is then held up with the hoop and handle down and the mosquitoes allowed to go up into the "top" of the bag. The killing tube is then introduced through the ring and placed over individual specimens. Considerable damage to the specimens or loss of material may result from this technique if too many individuals are captured at one time. In such cases an alternate method is to drive all the specimens into the apex of the bag by swinging the net carefully a few times, quickly pinching off the top with the specimens with the fingers and introducing this portion of the net into a wide-mouth killing jar, closing it over the upper part of the net with the lid of the jar.

Resting mosquitoes are readily picked up into an aspirator one or a few at a time and then transferred to a killing tube. It is always preferable to capture mosquitoes with an aspirator but it may not be practicable when very large numbers are encountered.

It is also possible to place a killing tube directly over a resting or biting specimen but to do this several killing tubes have to be used in relay and they may have to be recharged repeatedly.

KILLING AND STORING. The most satisfactory killing tubes are charged with chloroform but other killing agents may be used (see GLOSSARY). The killing tubes should be used exclusively for mosquitoes and should always contain strips of dry absorbent paper (tissue paper or paper toweling). When these strips get damp they must be replaced with fresh dry ones.

The specimens must be removed from the killing tubes within 10 minutes after being introduced into the tubes. The contents of the tubes are emptied into a plastic vial or plastic cup (depending on quantity of material) containing tissue paper or paper toweling on the bottom and more tissue paper is added on top of the specimens. A separate plastic vial or cup is used for every collection number which is written in grease pencil on the outside as well as in pencil on a slip of paper placed within. A small portion of the paper toweling or tissue is slightly moistened to keep the specimens soft and relaxed; the lid of the container must be carefully secured. It is best not to put the specimens into pillboxes in the field; this should be done at the end of the day in the laboratory or living quarters (see chapter on KILLING AND PRESERVATION).

ISOLATING FOR OVIPOSITION. A number of species of mosquitoes which are very common as adults are very seldom encountered as larvae and pupae and the immature stages and breeding sites of some of these are not even known. This is particularly true of the genera Aedes and Psorophora in Middle America. To obtain the immature stages of these it is necessary to isolate live individual females collected in the field, induce them to oviposit and to rear all the stages from the eggs (see PROGENY REARINGS in the chapter on REARING AND SORTING). It is also frequently desirable to study the range of variation in clones of important species using this technique for obtaining material.

Gravid or blooded females may be found in the field and should be isolated 1 to an oviposition vial. Some landing or biting females should be captured in oviposition vials after they are allowed to engorge to repletion. The cotton in the oviposition vial should be saturated with water but not contain free water and the vial must be kept out of direct sunlight. Cover the vial securely with the plastic lid and mark the outside with the collection number in grease pencil.

COLLECTING AND RECORDING. Collect wherever mosquitoes are encountered but pay particular attention to situations showing the edge effect, water effect and human effect, discussed in the preceding chapter. Record all the data for every collection on the standard record form. First enter the locality and general information in the top section of the form as indicated in the preceding chapter. Usually several collections will be made in one locality. A separate collection number (lot) is assigned to every collection in a specific site, of a specific type, at a specific height above ground, on a specific host or bait, and at a specific time of capture (see below). When, because of unusual conditions, a composite collection is made, the circumstances must be explained in the section on REMARKS. A collection of adults for progeny rearings must be assigned a number distinct from any general adult collection made at the same time and place.

Record all the appropriate items on the collection form in the section on GENERAL ENVIRONMENT for every collection as indicated in the preceding chapter. Then check every item in the section on ADULTS as discussed below. These two sections serve as guides to the selection of places and methods of collecting adults in the most productive environments. Any unusual conditions such as rain, stormy weather, etc. should be recorded under REMARKS.

1. Site. The exact situations must be indicated, not the general environment. Describe also such things as the size of the crabhole, type of platform in a tree, condition of the host or bait and so on.

2. Type. Indicate the type of collection by underscoring or circling one and only one of the items listed. The most common collections are biting-landing collections with the collector or an assistant serving as bait; it is

usually not possible to distinguish between landing and biting collections and they are therefore regarded as one type. For these collections a protected spot should be selected for a team of two men, one serving as bait, the other one capturing the landing or biting mosquitoes; the bait will attract more specimens if the shirt is removed or trouser legs or sleeves are rolled up. Excellent collections can be obtained by a collector working alone. Different species of animals may also be used as bait for biting-landing collections. Use an aspirator for capturing specimens and spend at least 30 minutes at a collection site. If mosquitoes are extremely numerous it may be necessary to use the net and wide-mouth killing jar technique. Nets are very handy also in the case of many sabethines which are frequently very chary about landing and biting.

Swarming mosquitoes, consisting primarily of males, are sometimes encountered over bushes or various projecting objects, including the head of a collector. Such swarms should be captured with a net, in toto if possible, and careful notation made of all circumstances under the site.

Resting collections are made in a variety of habitats where mosquitoes normally concentrate during periods of inactivity. Almost any protected, cool, shady spot may serve as a resting place, but the most productive sites of concentrations of resting mosquitoes are: buttresses of trees, hollow trees, undersides of fallen trees, cavities in the ground, crabholes, animal burrows, caves, banks of streams, draws or ditches (particularly with overhanging vegetation or dripping water), sides of waterfalls, dense mats or stands of herbaceous or grassy vegetation, axils, bracts and pitchers of waterholding plants as well as on or under various structures such as bridges, culverts, drains, pipes, barrels, boxes, bottles, cans, in barns, privies and in and under houses. It is among resting mosquitoes that one will find the largest percentage of males and gravid and blooded females in the field. Search through all the different types of resting sites; a flashlight will be useful in many dark places. It is frequently necessary to disturb the site in some fashion to locate the mosquitoes. Blowing tobacco smoke into small cavities, such as crabholes, is helpful. Resting mosquitoes are generally collected individually with an aspirator, directly into oviposition vials or with a net. With crabholes, animal burrows and similar cavities in the ground, resting mosquitoes may be driven into a net placed over the hole and the bag held up by stamping around the hole or prodding into it with a stick or aspirator. The specimens are then picked up from the net with an aspirator or killed in a wide-mouth jar as indicated above. The exact resting place collected must be specified on the form under 1. Site. Every type of resting place must be given a separate collection number (lot).

Sweeping collections are essentially collections of concentrations of mosquitoes resting in relatively open situations in grassy or herbaceous vegetation or on shrubs, collected by sweeping a net above the vegetation after the mosquitoes are disturbed into flight by walking through or beating the vegetation. They usually consist of recently emerged specimens of both sexes resting in the vicinity of the breeding sites. The males of many species are most easily obtained in this manner before they disperse or die. The most productive areas for sweeping collections are along margins of streams, ponds and marshes as well as in the interior of swamps and marshes and also in the neighborhood of temporary pools in open situations as well as in wooded areas. Care should be taken that the net clears the vegetation in sweeping, that it does not get wet, and that only a small number of specimens is captured at one time.

The wide-mouth killing jar technique should be used for killing the specimens (see above) unless only a few specimens are captured when an aspirator and killing tube may be used. A portable screened chamber is useful for capturing mosquitoes resting in grass (Zulueta 1950).

The term light collections refers to captures made at night at an artificial light source of some kind. A variety of nocturnal and even diurnal species may be attracted to these lights and this type of collection often includes a large percentage of males not readily obtained by other methods. All types of electric lights, incandescent, fluorescent and ultraviolet ("dark light"), are effective attractants except for lights emitting longer wave lengths in the yellow and orange range. Collections should be tried at various stationary light sources in settlements, isolated houses and along highways. Mosquitoes should be looked for on horizontal and vertical surfaces from near the light source to a considerable distance away where relatively little light falls; some may be found flying around the light. Portable light sources (battery-operated electric lights; gasoline or kerosene lanterns) may be taken into the field and operated with a sheet placed under the light and another hung vertically a short distance from the light. Mosquitoes should be captured with an aspirator unless extremely numerous in which case a net should be used. Various kinds of light traps have been designed and widely used in many parts of the world, primarily in north temperate regions. Light traps in general have not proved to be very effective in most tropical areas for unknown reasons. Attempts should be made to develop more effective light traps for these areas for they could be extremely helpful in obtaining certain nocturnal and crepuscular species. Not all light traps are suitable for collecting mosquitoes for taxonomic studies as the specimens may be in very poor condition in such traps as the most widely used "New Jersey" light trap and its various modifications (Mulhern 1953). The most promising and potentially best suited light trap for taxonomic studies is the "CDC miniature" battery-operated light for the capture of live specimens (Sudia and Chamberlain 1962). All collecting at lights or in light traps is strongly influenced by atmospheric conditions and by moonlight. It is important therefore to make for every collection a notation as to the exact conditions under which captures are made as well as the type of light or light trap used. Each different collection should be given a separate number as with all other types of collections.

Trap collections are restricted here to captures with all types of traps except light traps. Many different animal-baited traps have been designed but the most useful ones for mosquito collecting in the tropics are the Shannon trap (Shannon 1939, Forattini 1962: 603-607), the Magoon trap (Magoon 1935, Bates 1944, Russell et al 1963: 298), the dawn trap (Shannon 1943, Earle 1949) and the Trinidad no. 10 trap (Worth and Jonkers 1962). The captures in some of these traps consist primarily of blooded females that do not make good museum specimens but which are very important as parent females for progeny rearings. A sample of females should be isolated in oviposition vials and the remainder killed and preserved. Other traps do not allow the females to feed on the bait and material caught in these traps should be killed and preserved directly. Traps baited with "dry ice" (CO_2) have been used successfully in several areas but are not practical for field use in most places in the tropics. Recently the unbaited portable Malaise trap (Malaise 1937: 148-160; Gressitt and Gressitt 1962; Townes 1963) has been used very successfully for sampling many different types of flying insects and is particularly efficient in capturing

Diptera. It should be tried and adapted for mosquitoes and may capture nocturnal as well as diurnal species. With all trapping the selection of the site for the trap is all important and such information should be included on the collection form together with an indication of the specific trap used.

3. Height of collecting site above ground should be carefully recorded in meters for all collections. Biting-landing and sweeping collections with collectors standing at ground level are recorded as 0 meters. For biting-landing collections made above ground level, as on a platform on a tree, the exact height above ground should be given. For swarms, resting, light and trap collections the height at which mosquitoes are found should be entered.

4. Host or bait. The type and number of hosts or bait used in biting-landing and baited-trap collections should be indicated.

5. Time of capture. The inclusive period during which the collection is made should be entered, preferably on a 24-hour clock system. The time of operation of a trap should be similarly indicated.

COLLECTION OF IMMATURE STAGES

EQUIPMENT. The basic equipment and supplies needed for the collection of immature stages are: (1) dippers, (2) aquatic and dip nets, (3) mosquito pumps and siphons, (4) pipettes and medicine droppers, (5) plastic, enamel or porcelain sorting bowls, (6) plastic pans and buckets, (7) plastic cups with lids, (8) plastic vials with lids, (9) plastic bags, (10) a water can or other large plastic container with clean fresh water, preferably rainwater, (11) box, carton or tray to hold filled plastic cups and vials and (12) a collecting bag fully equipped as indicated in the GLOSSARY.

COLLECTING AND RECORDING. Because of the much greater percentage of species that can be collected as immature stages as compared to adults the emphasis in taxonomic surveys should be placed on the collection of immature stages which can then be reared with relative ease in the laboratory to provide definite association of both sexes and all stages.

The immature stages should be collected with great care to prevent injury and should be provided with a sufficient volume of water and fine debris from the original breeding site to insure adequate food supply for rearing. Larvae and pupae are removed from the breeding site with the appropriate tool such as a dipper, aquatic net, dip net, pipette, siphon or pump. Do not neglect to collect small larvae. All immature stages are placed with ample water from the breeding site into a plastic cup, sorting bowl, pan or bucket until the desired number is obtained. Large debris is removed from the container and the sediment (if present) is allowed to settle. Because pupation and emergence of adults often occurs during midmorning and midafternoon while collections are being made or transported it is very important to isolate in individual vials the desired number of full grown fourth instar larvae (identified by dull opaque bodies) and dark pupae. These individual vials are capped and carefully marked on the outside with the collection number (lot) in grease pencil. The remainder of the collection is carefully searched for predators and for carnivorous mosquito larvae; for recognition of carnivorous larvae see section on SPECIAL REARINGS in the chapter on SORTING AND REARING. The latter must also be isolated in individual vials. If a large amount of water is present, pour out the excess through a fine-meshed dip net, invert the net and dip it into the

water which is retained in the container. The final collection should be placed into one or more plastic cups about three-quarters full of water and containing no more than about 200 larvae and pupae each. Cover carefully the plastic cups with lids and mark each cup and lid on the outside with the collection number in grease pencil.

It is very important to wash carefully all pipettes, nets, dippers, etc. between all collections so that no transfer of larvae, pupae or eggs will take place. It is obvious that some records of mosquitoes from unusual breeding sites are due to inadvertent contaminations of this type.

Every collection from a specific individual breeding site should be assigned a separate collection number. Record all the data for each collection on the standard collection form indicating all the appropriate data for each of the items in the section on IMMATURE STAGES. The data to be entered are discussed below under BREEDING SITES, HEIGHT ABOVE GROUND, WATER, VEGETATION and BOTTOM. Assign a number to the collection, enter all the data on the top of the card for locality and general information and in the section on GENERAL ENVIRONMENT. All this information must be given for every collection even when several are made in the same locality.

In the section on BREEDING SITES below, all the principal types of breeding sites utilized by mosquitoes are listed and this section will serve as a guide to specific places to look for immature stages.

COLLECTING EGGS AND DRY MATERIAL. A rich but neglected source of mosquitoes in general surveys are the eggs occurring in natural habitats. For certain species, particularly in the genera Aedes and Psorophora, this forms the most dependable means of obtaining larvae, pupae and adults, especially during the dry season. An effort should be made therefore to collect eggs in the field and to rear them in the laboratory. In addition, it is very desirable to obtain more information about the eggs of mosquitoes since very little is known at present, particularly about the eggs of species breeding in the leaf axils and flower bracts of plants. It may be advisable to preserve some of these eggs in the field (see chapter on KILLING AND PRESERVATION).

In those species utilizing permanent or semi-permanent collections of water on the ground, eggs are laid usually on the water surface but sometimes are attached to vegetation. In the former case, eggs are usually collected together with the larvae and pupae and will be included in the collection of these stages if a dip net is used to filter the water and concentrate the debris from the breeding site. However, a special effort may be made to locate egg rafts or individual eggs of anophelines and to isolate these in vials for rearing. These eggs may be picked up with a camel's hair brush or a strip of absorbent paper such as paper toweling or filter paper. Certain species of Mansonia attach masses, rosettes or ribbons of eggs to emergent or floating vegetation or various objects at or below the water surface and sometimes even to the roots of aquatic vegetation (see Horsfall 1955 for specific sites). Wherever Mansonia adults are collected, a search should be made for eggs in various permanent bodies of water nearby. The egg masses should be removed together with a piece of the vegetation and taken to the laboratory for rearing.

Species utilizing primarily temporary ground waters for breeding (Psorophora and several subgenera of Aedes) lay eggs, usually individually, on moist soil above the water level or in depressions without water at the time but where flooding may subsequently occur. Larval development is usually very rapid in these species and generally immature stages will not be found when only females are encountered in the field. In such situations an attempt should be

made to collect eggs around the edges of depressions where water may have been standing. Samples of the upper inch or less of the soil should be gathered with a trowel or small shovel and placed in plastic bags or plastic cups to be flooded later with water (see chapter on SORTING AND REARING). Eggs of these species may also be collected during the dry season from likely areas, particularly where adults may have been noted during the rainy season. All low areas subject to periodic flooding should be checked as well as margins of semi-permanent or even permanent ground waters subject to periodic fluctuation. The presence of eggs in the soil may be checked in the field by flooding a sample of the dry soil in a plastic cup; if eggs are present larvae will hatch usually within minutes. Eggs of different species may be laid at different levels of soil humidity so that a transect should be made from the lowest to the highest point in the depression.

Many species utilizing treeholes and possibly other plant container habitats (leaf axils, flower bracts, coconuts, bamboo, etc.) lay eggs above the water level in the breeding site. The eggs of some of these species can withstand desiccation and may survive long dry seasons when no water at all is found in the breeding site. After collecting larvae and pupae in such habitats, the sides of the treehole or other container habitat should be washed down several times with water to dislodge any eggs that may be present. A considerable amount of the debris at the bottom of the container should always be included with the water taken for rearing. During the dry season one can usually identify treeholes that may serve as breeding sites at the appropriate time by a water stain below the lip of the hole. Debris from the bottom and scrapings from the sides of such dry treeholes should be collected in plastic bags or plastic cups for rearing. Dry debris from the basal axils of agave and some yuccas has also yielded eggs of species of Aedes and it is possible that debris from leaf axils and flower bracts may also harbor viable mosquito eggs.

All egg collections should be treated exactly like collections of other immature stages, given a separate collection number for every specific site and all the information in the section on IMMATURE STAGES on the collection form filled in the same way. In addition a notation should be made under REMARKS indicating the nature of the collection.

TRANSPORTING COLLECTIONS. The various containers with immature stages or adults collected in the field should be placed upright in boxes or cartons. Usually no difficulty is encountered and little or no mortality occurs if some care is taken in transporting live mosquitoes in field vehicles. First, the containers should be placed away from direct sunlight and excessive heat; wet towels may be placed over and around the containers when high temperatures are encountered. Second, drive carefully avoiding high speeds, sudden stops and excessive bouncing. Third, stop periodically (every hour or so) for 10 to 15 minutes and check through the collections. Open the plastic cups to relieve any pressure that may have built up and reseal the containers. This is particularly important when changing altitude rapidly and at such times should be done more frequently. Fourth, take care of any adults that may have emerged.

Various special methods have been used for transporting larvae and pupae but these are usually not essential if the above suggestions are followed. Collections have been transported in a passenger car on trips of a week or longer over very rough roads without special methods with practically no loss of material.

BREEDING SITES. The list of breeding sites on the collection form provides a guide to the major types of specific habitats utilized by the immature stages of mosquitoes. All the types should be checked in every locality being surveyed. A separate collection number should be assigned to each specific type and separate collection forms filled out for each one as indicated above under COLLECTING AND RECORDING. In the present section all the different types are defined and discussed in the order in which they are listed on the form and suggestions are made for special collection methods that can be used to best advantage. The list of sites begins with the most generalized habitats on the ground and proceeds to the most specialized living plant containers. Any unusual habitat not covered in the list should be specified along with all explanatory remarks for other items in the section on REMARKS.

1. Pond, lake. This category includes all types of permanent or semi-permanent bodies of standing water, natural or artificial, with a considerable expanse of open area in the center. The distinction between pond and lake is largely one of size. A lake is larger and usually fed by one or more definite streams while a pond is small and largely fed by springs or seepages. Most lakes are named on topographic maps while ponds have only local names if any. Collections should be made at various sites and in different types of vegetation around the periphery and also if possible in the central part. Each of these collections should be given a different number and a note made under REMARKS to indicate the exact site for each in addition to specifying the different conditions of water, vegetation and bottom. Immature stages are most easily located by trampling the vegetation and muddying the water. A dipper or a net can then be used to pick up the larvae and pupae as they come up to the surface. Along abrupt banks and where the vegetation grows in hummocks the immature stages are generally concentrated along the edges of the vegetation; here a quick plunge or a slow gradual depression of an inclined dipper against the edge will capture many specimens. In more open situations sweeping through the water with an aquatic net will be more rewarding.

No difficulty will be encountered in locating and collecting the immature stages of most common and well known typical mosquitoes of the genera Anopheles, Bironella, Uranotaenia, Culex, Aedeomyia, Hodgesia, Culiseta, Ficalbia and occasionally Aedes. The general technique of handling these has been discussed briefly above under COLLECTING AND RECORDING; the concentration technique is particularly important to obtain large numbers of immature stages and should always be used. However, certain forms common in lakes and ponds require special attention to recognize, find and/or handle. These are discussed in the following paragraphs.

Mansonia larvae and pupae will be found below the water surface attached to floating vegetation or to stems and roots of emergent plants. To locate the breeding sites of some of these, an aquatic net may be swept under the vegetation with a lifting motion and the contents emptied into water in a large pan or bucket. For other Mansonia species either a bucket may be placed carefully under a mat of floating vegetation and lifted out with the vegetation, or clumps of floating vegetation may be carefully lifted and placed into a pan or bucket of water. In either case the vegetation is then agitated vigorously to dislodge the larvae and/or pupae and carefully examined piece by piece to locate them. The debris settling at the bottom of the pan or bucket should also be carefully searched for larvae and pupae. At the time of emergence, Mansonia pupae float up to the water surface where they rest motionless in a horizontal position

apparently lifeless and quite unlike ordinary mosquito pupae and resembling the pupae of chironomid midges. Breeding sites of Mansonia can be located sometimes by finding large numbers of cast pupal skins. Live Mansonia larvae and pupae should be placed in plastic cups or individual plastic vials provided with small discs of wet strength paper floating on the surface (see section on SPECIAL REARING in chapter on SORTING AND REARING). Dark pupae should be isolated in individual vials with a moist strip of paper toweling and no free water. Some species of Ficalbia also attach to vegetation and should be treated in the same manner as Mansonia.

Chaoborus and Sayomyia larvae and pupae are frequently found in deeper water in the more open situations away from shore. They should be collected by sweeping rapidly a large aquatic net at several depths and also by bringing up mud from the bottom. The net is then everted and washed in a shallow pan of water. The larvae and pupae are practically transparent and difficult to locate even in a pan except after the sediment has settled when they can be recognized by their jerky motion as they float horizontally near the bottom of the pan. Although carnivorous, Chaoborus and Sayomyia larvae may be left together in a plastic cup as they do not feed on other mosquitoes.

Corethrella and Lutzomiops larvae and pupae also require special attention. The larvae resemble short stubby Anopheles or Uranotaenia and rest parallel to and just under the surface film. Some species are very easily disturbed and when alarmed will sink and remain for long periods in the flocculent muck at the bottom. The pupae float motionless on the surface film in a horizontal position and resemble seeds or chaff. As with Chaoborus it is not necessary to isolate individual specimens of Corethrella and Lutzomiops since they do not seem to be cannibalistic and probably also feed primarily on the larger microorganisms in the larval habitat.

Immature stages of Dixinae are commonly found in vegetation around ponds and lakes and in other ground water habitats but are seldom collected because they are not recognized as relatives of true mosquitoes. The larvae move backwards with a rapid looping motion of the bodies apparently on top of the surface film and, when coming to rest against vegetation or shore margin, bend into an inverted U with only the head and tail in the water and most of the body completely out of water. They frequently crawl out of the water completely but are of course surrounded by a film of water. They can be dislodged from such situations by quickly pouring water from a dipper or splashing water on the shore or vegetation and then are collected with a dipper or net. The pupae are found with the larvae resting against edges with the long abdomen hanging straight down. After capture Dixinae immatures are handled like true mosquitoes.

2. Ground pool. Included in this category are all kinds of collections of standing water on the ground that are primarily of a temporary nature, whether natural or artificial. Some of the larger ground pools may be semi-permanent as in the case of borrow pits around roads and railroad embankments, along irrigation dikes and in irrigation overflows. Ground pools may also be formed in depressions after water recedes from overflows of ponds, lakes and streams. A large ground pool is considered to be one 5 meters or more in the largest dimension and a small ground pool of less than this diameter. A very common type of small ground pool are ruts made by vehicles in dirt roads. Ground pools should be looked for in open areas, in grassy areas and in second growth wooded areas; each of these general environments will yield different species of mosquitoes. A special category, 5. Flooded forest, is considered separately below because it is seldom collected and may have species of special

interest. Belonging in the same category with ground pools are also 3. Animal tracks.

Collections in ground pools are easily made with dippers, dip nets, aquatic nets or even directly with pipettes. The mosquito fauna consists primarily of species of Aedes and Psorophora but in the larger and in semi-permanent pools species of Anopheles, Culex, Uranotaenia, Culiseta, Chaoborus, Sayomyia, Corethrella, Lutzomiops, Dixinae and even Mansonia may be found. For the latter four groups see the discussion under 1. Ponds, lakes above.

3. Animal tracks. Tracks made by animals in low lying and marshy areas in the open as well as in woods are productive breeding sites particularly during the rainy season. The immature stages may be captured directly with a pipette or with a dipper or small dip net. The latter is particularly useful as it captures specimens more quickly and concentrates food. Although the mosquito fauna of animal tracks consists primarily of temporary pool breeders (Aedes, Psorophora), at times it includes species normally breeding in permanent habitats (Anopheles, Culex, Uranotaenia, etc.).

4. Swamps. As understood here this category includes marshes as well as swamps of all types, i.e. permanent or semi-permanent areas of wet ground, more or less uniformly covered with standing water but without extensive areas of open water. A wide variety of special types of sites are usually present in swamps and all the different situations should be checked by collecting along a transect from the edge to the center. All the groups mentioned under 1. Pond, lake are usually represented in swamps, frequently by species peculiar to these habitats. Of particular interest are some mosquitoes that breed in areas so choked with vegetation that no water at all is apparent until one sinks into the mat overlying the water. Areas of this type with low vegetation, found around the periphery of some swamps are the marshy depressions mentioned on the collection forms.

Collecting in swamps is often difficult because of the density of the vegetation and it is often necessary to cut down the tall vegetation with a machete, trample the remaining stubs and muddy the water before the immature stages can be located. Frequently peculiar species will be found localized in small pockets of water and at the edges of hummocks of vegetation. Swamps are particularly rich in species of Mansonia.

5. Flooded forest. A common temporary ground pool habitat seldom collected is the flooded forest floor. In the Solomon Islands and New Guinea the larvae of several species of very common Aedes previously unknown in the immature stages were found in this type of habitat. Frequently the water is very shallow and intermittent; apparently some species complete their development during a series of floodings, surviving in the mud or decaying vegetation as larvae or pupae between floodings. This type of habitat should be thoroughly investigated as a possible source of species of Aedes and Psorophora still unknown in the immature stages.

6. Seepages and springs. Sources of flowing water serve as breeding sites for many species of mosquitoes which may be restricted to this type of habitat. The following forms are frequently encountered: Dixinae, Anopheles, Bironella, Chagasia, Uranotaenia, Culex and Culiseta. Depending on the depth of the water, pipettes, dippers and dip nets may be used to capture the immature stages. Springs and seepages in caves are of particular interest and should be investigated more thoroughly than they have been in the past.

7. Wells. These artificial sources of clean fresh water harbor a mosquito fauna very similar to that found in seepages and springs (see above). Species

of Chaoborus and Sayomyia may also be present. Here dip nets and even aquatic nets may be used in addition to dippers for capturing the immature stages. The sides of wells frequently serve as resting sites for adult mosquitoes.

8. Streams. Streams of all sizes and types should be investigated thoroughly for they serve as breeding sites for many species of Dixinae, Anopheles, Bironella, Chagasia, Uranotaenia, Culex, Aedeomyia (occasionally), Hodgesia, Culiseta, Ficalbia and sometimes Mansonia. Most immature stages will be found in the vegetation or against the banks of the stream but species vary a great deal in their preference with respect to strength of the current and some may even be found along the edges of boulders in the swiftest part of the current. Pools on the sides of a stream or in a drying stream bed are also very productive. A special type of site which often contains concentrations of immature stages of many species will be found upstream from blocks across the stream where flotage and debris accumulate.

9. Ditches and drains. These artificial channels of flowing water provide habitats very similar to those found in some types of streams and harbor a very similar but more restricted mosquito fauna. The smaller drains resemble seepages and springs and are often contaminated with organic domestic wastes. Canals of all types should be included in the category of ditches and drains.

10. Fountains, gutters. These are artificial collections of running water with concrete bottom and usually without conspicuous large vegetation. A limited but significant mosquito fauna of domestic, quasidomestic and some wild species utilize this habitat, particularly species of Culex and Anopheles. The immature stages may be captured with dippers, dip nets or pipettes.

11. Crabholes. Holes made by land crabs (Gecarcinidae) and fiddler crabs and related forms (Ocypodidae) are normally utilized for breeding and resting by a large number of species of the genera Deinocerites, Culex and Aedes and a few species of Uranotaenia. The genus Deinocerites and several Old World subgenera of Aedes (notably Cancraedes, Geoskusea and Levua) are practically restricted to crabholes. Representatives of other genera and subgenera may be occasionally found in crabholes. Some crabhole species are sometimes found in treeholes and artificial containers and not infrequently in temporary ground pools in an area where crabholes are present. There appears to be little or no specificity of association of a given species of mosquito with a particular species of crab. However, crabholes of different size and in different environments harbor different species of mosquitoes, although two or more species are often found in one hole. Consequently, a separate number (lot) should be assigned to the material obtained from each individual crabhole. Crabholes containing mosquitoes have been found in and along the edges of mangrove swamps, on the banks of streams, rivers and lakes near the sea and even inland (Lake Valencia in Venezuela) and from hillsides. Generally the water in the crabholes is fresh or only slightly brackish and is not subject to flooding by tides. Rainfall affects markedly the suitability of crabholes for mosquito breeding and this undoubtedly accounts in part for the seasonal differences in the distribution of crabhole mosquitoes. Therefore collections should be made at all time of the year and from all types of crabholes.

Some crabholes are shallow and the immature stages in these can be readily seen at the water surface. In the majority of crabholes, however, the water surface is from a few inches to 2 meters or more below the ground surface, i.e. at the level of the water table, or approximately at the level of the water

in the nearby swamp, river or lake. Not all crabholes contain immature stages, usually only those in which adults are resting. The presence of adults can be determined by blowing tobacco smoke, prodding with a stick or aspirator or stamping on the ground if it is solid. These adults should be collected with an aspirator as they return to rest in the hole, as they invariably do within seconds, or if many are present by inverting an aerial net over the hole and driving them up into the apex of the net. These adult catches should be preserved and given their own collection number but with a crossreference to the collection of immature stages from the same hole.

The easiest way to capture the immature stages is with a mosquito pump. The end of the rubber tube of the proper diameter attached to the pump chamber should be carefully inserted into the crabhole until it hits the bottom. It should then be raised slightly so that it does not become clogged with sand or debris. The one-way rubber bulb attached to the other opening of the pump chamber should then be squeezed repeatedly until the chamber is filled or all the water drawn up. Do not overfill the pump for this may clog the bulb. The rubber tube is then withdrawn from the crabhole and the rubber stopper connecting it to the chamber removed from the pump as well as the bulb together with its fitting. Now one or more plastic cups, as needed, are filled about three-quarters full of water from the pump chamber using the large opening. The rest of the water is poured through a dip net bag to recover the immature stages and to concentrate the food and debris. The bag should then be inverted and dipped several times into the water in the plastic cups to wash off all the material. Crabholes become quickly refilled with ground water and should be pumped out repeatedly until all the immature stages are recovered.

A record should be made of the size of the crabhole; a large one is arbitrarily designated as being 5 centimeters (2 inches) or over in diameter for the purpose of our records. The species of crab should be determined if possible and all information on the condition of the crabhole should be recorded.

Other animal burrows or cavities in the ground partially filled with water may serve as breeding sites for mosquitoes, as has been found to be the case for a number of species of Aedes in Australia (Marks 1957). Any potential breeding sites of this type should be investigated with the aid of a mosquito pump.

12. Rockholes. Holes in various types of rock in a variety of locations serve as specific breeding sites for a number of mosquitoes of the genera Anopheles, Culex, Uranotaenia and especially Aedes. These sites may also be utilized by some general ground water breeders in the same genera as well as some Dixinae, Bironella, Culiseta and Haemagogus. The most common types of rockholes are in various types of rocks (sedimentary, igneous or metamorphic) along the sides of streams, and pot holes in boulders in or around the stream bed. Similar types of holes with quite a different fauna are found along the seashore. Holes in volcanic or coral rocks in various locations, often away from streams or the seaside, may also serve as breeding sites for mosquitoes. The immature stages are readily captured in all these sites with a pipette, dipper or dip net depending upon the size of the hole. Occasionally a pump may be useful to empty a deep narrow rockhole. Since the food is frequently rather limited in these habitats the contents of rockholes should be stirred up vigorously after the immature stages are captured and added to the water in the plastic cup after filtering through a dip net bag. Large debris, sticks, leaves and so on, should be removed however.

13. Artificial containers. Many species which normally breed in natural containers such as treeholes, bamboo, fallen plant material and apparently even those that breed in leaf axils and flower bracts may utilize various types of artificial containers. Some ground water breeders may utilize large artificial containers. When conditions are suitable, populations in artificial containers may be extremely high.

Any man-made object that will hold water may serve as an artificial breeding site, ranging from water tanks, disused swimming pools and boats to tires, tin cans, bottles, cups and saucers, flower vases and canvas. Collections from different containers should be kept separate and given individual collection numbers. It is important to note the size of the container (a large one is arbitrarily defined to container 10 liters (2 and one half gallons) or more of water) and all the conditions indicated on the collection cards within and outside the container since this information may help to locate the natural breeding sites of some species collected to date only in artificial containers.

With small containers, the immature stages may be poured directly into a plastic cup after stirring the contents. When more water is present the immature stages and larvae may be concentrated by pouring most of the water through a dip net bag and washing this off into a plastic cup. For the very large containers, dippers or dip nets should be used and care must be taken to take along some food and debris with the water.

14. Treeholes. The term treehole is used here for all types of water accumulations in standing and usually living trees, primarily in rotholes but also on the surface of the trunks, buttresses or aerial roots. The deep rotholes, often with very narrow horizontal openings, are the most specific type. A very large number of species are restricted to breeding sites in treeholes, including representatives of Anopheles, Uranotaenia, Culex, Culiseta, Ficalbia, Orthopodomyia, Eretmapodites, Armigeres, Heizmannia, Aedes (many subgenera), Haemagogus, Malaya, Maorigoeldia, Tripteroides, Trichoprosopon, Wyeomyia, Sabethes, Toxorhynchites and Corethrella. Many additional species may occasionally use large treeholes for breeding. The water in treeholes is usually temporary, but at least some types of treeholes (tree wells) appear to contain water throughout the year even in areas with very limited rainfall. Treeholes do not seem to be abundant in areas where epiphytes are common, probably because the latter become established in rotholes soon after they appear. However, it is possible that some treeholes are hidden between epiphytes in tree crotches or at the base of branches. Very little information is available about the specificity of the association of mosquitoes with a particular species of host tree but it is likely that a considerable degree of specificity for at least some forms will be shown eventually as is evident now with bamboo which is accordingly treated separately below.

Relatively few species of plants harbor treeholes suitable for mosquito breeding and considerable time has to be spent searching for these. All parts of the tree should be carefully examined. Some rotholes have very narrow openings which appear to form at the point of origin of a branchlet on a large branch or main trunk. Frequently a rothole can be spotted on a tree by a stain indicating overflow of water from its rim. The presence of biting adults of species known to use treeholes is also indicative of treeholes in the vicinity.

Water in treeholes is frequently so dark that it is difficult to determine the presence of immature stages by direct inspection in the breeding site or even by a careful search in the water removed with a pipette into a deep container.

The following technique is suggested for all treehole collections in order to recover as much of the material as possible, including eggs. If the opening into the treehole is very small it should be enlarged with a knife to the width of the cavity below. The sides of the hole above the water line should be scraped and washed with a strong stream from a pipette filled with treehole water.

If the treehole is small (arbitrarily defined as one containing less than 1 liter (quart) of water) an ordinary collecting pipette or preferably a battery pipette should be used to remove all the water into a container of a suitable size. Clean fresh water is then poured into the treehole, the contents thoroughly agitated and all the water and debris removed with the pipette and added to the original collection. Some of this water is used to fill one or more plastic cups three-quarters full. The remainder of the collection is now poured through a dip net bag for concentration, the water itself being saved to refill the treehole. To determine the presence of immature stages or to sort them, the concentrate in the dip net bag is transferred to a plastic bowl or pan containing clean fresh water by immersing and agitating the inverted bag. Large chunks of debris, leaves, sticks, etc. are picked up, washed in the bowl and discarded. After the inspection or sorting is completed, the water and all the material from the bowl is poured through a dip net bag again and the concentrate washed into one or more of the plastic cups containing some of the original water from the treehole.

If the treehole is a large one (containing 1 liter (quart) of water or more) a small or large mosquito pump should be used to remove the water employing the technique of pumping described above under 11. Crabholes. The rest of the procedure outlined above should be followed to concentrate the collection except that some of the excess water from the first pumping may be used to wash the treehole for the second pumping. Upon completion of the collection all the excess water should be returned to the treehole and fresh clean water used to fill it.

Every collection from each individual treehole should be given a separate collection number (lot) and notation made on the collection form of all data pertaining to each. Occasion may arise to collect in the same treeholes at a later date, particularly if an unusual form is discovered. The scientific or common name of the tree should also be noted.

As indicated above in the section on COLLECTING EGGS AND DRY MATERIAL debris should be collected from dry treeholes if there is any indication that they may have held water at some time.

15. Fallen trees. Accumulations of water in depressions on fallen trees are utilized by a variety of species for breeding. Apparently very little specificity exists for this type of habitat but accurate information is needed on this point. Both container and ground water breeders have been noted from fallen trees. The techniques of capturing discussed under treeholes and large artificial containers should be used.

16. Bamboo. The mosquito fauna of bamboo is essentially similar to that of treeholes in general on the generic level but it is evident that there is a great deal of specificity for bamboo by many species, groups and subgenera. Included with bamboo should be other arborescent or large grasses commonly called reeds (Phragmites). In general in any one area the specific bamboo breeders are to be found only in the bamboo indigenous to that area. Two markedly different types of habitat in bamboo are apparent: (1) cut or broken bamboo whether standing or fallen, provides sites for the general container breeders and the

less specialized bamboo fauna and (2) internodes of uncut bamboo with small or medium-sized holes made primarily by insects (Macdonald 1960: 136-146) harbor a specialized and unique fauna. Species of the following genera are known to breed in bamboo: Anopheles, Uranotaenia, Culex, Orthopodomyia, Eretmapodites, Armigeres, Heizmannia, Aedes (several subgenera), Haemagogus, Topomyia, Tripteroides, Trichoprosopon, Wyeomyia, Limatus, Sabethes, Toxorhynchites and Corethrella.

Collecting in cut, broken or fallen bamboo is readily accomplished with a battery pipette or small mosquito pump as in treeholes (see). For uncut internodes the entrance hole or holes may be enlarged to introduce the rubber tube from a small mosquito pump. Alternately the culm may be cut above the hole but this destroys the breeding site and preferably this should be avoided. A careful search should be made for uncut internode breeding sites in bamboo shoots and culms of different age and with different size entrance holes for there may be great specificity in the utilization of sites in this respect by different species of mosquitoes. Collections from each internode should be given separate collection numbers (lot) and all the specific information about each recorded carefully.

Since breeding sites in uncut bamboo internodes are difficult to find it is suggested that artificial "worm" holes be bored with a brace and bit in culms of different age. These artificial breeding sites should be marked with a wax pencil and checked periodically for breeding.

17. Animal containers on ground. A few species of mosquitoes have been found breeding in water collections inside the empty shells of land snails in the Old World tropics but apparently this potential habitat has not been checked extensively in the New World. Other animal remains with accumulations of water should also be checked. Whenever the shell or remains can be identified the name of the animal should be given.

18. Fallen leaves, fronds, spathes. Large leaves, fronds and flower spathes (particularly palms) lying on the ground accumulate water which is used as breeding sites by a large number of species of the genera Culex, Uranotaenia, Zeugnomyia, Armigeres, Aedes (several subgenera), Eretmapodites, Tripteroides, Trichoprosopon and Limatus. Immature stages from such sites are easily collected with a pipette or by pouring the contents directly into plastic cups after thoroughly agitating the water to wash off any eggs that may be stranded above the water surface. With the larger fronds and spathes the contents should be concentrated first in a dip net bag as with treehole material (see). Care should always be taken to treat the contents of each site as a separate collection and a note made of the exact nature of the container, if possible indicating the species of plant.

19. Fallen fruits, nuts, rinds. Many kinds of fruits and nuts with fibrous, woody or stony layers fallen on the ground frequently collect water which is known to be used for breeding by a large number of species of the genera Uranotaenia, Culex, Armigeres, Aedes (several subgenera), Haemagogus, Tripteroides, Trichoprosopon, Wyeomyia, Limatus. The most common breeding sites are in coconut shells and coconut husks. The water in the plant container should be agitated and poured into plastic cups. It is advisable to wash the container with fresh clean water and to save this also. The type of container should be noted and a careful record made of the water condition, particularly with respect to the amount and type of organic matter present.

20. Attached fruits. Occasionally water collects in opened nuts or pods which remain attached on trees. Rat-eaten cacao pods are known to breed mosquitoes in the South Pacific islands and possibly similar breeding sites will be found elsewhere. The contents should be agitated and poured off into a plastic cup, the inside of the nuts thoroughly washed and the wash water added to the original contents. The type of fruit should be specified as well as a notation made about the nature of its contents.

21. Leaf and frond axils. Many species of monocotyledonous plants, both herbaceous and woody, hold for long periods of time a considerable quantity of water in the leaf or frond axils and petiole bases. These water holding plants have a unique and very large mosquito fauna with representatives of the majority of genera including Anopheles, Uranotaenia, Culex (several subgenera), Ficalbia, Orthopodomyia, Eretmapodites, Armigeres, Aedes (several subgenera), Malaya, Topomyia, Tripteroides, Trichoprosopon, Phoniomyia, Wyeomyia, Limatus, Sabethes, Toxorhynchites and Corethrella. Nearly all the species utilizing these sites are restricted to them. Only a few species, particularly on small islands, may utilize other container sites and a few introduced container breeders occasionally invade leaf axils. The specificity of the association of a mosquito species with a host plant species varies considerably but has not been thoroughly determined. It appears to be greatest with host plants which secrete a substance into the axil water which makes it slimy or gelatinous.

Any plant containing water in its axils should be examined for mosquito breeding but especially members of the following groups: pandanus family (Pandanus, Freycinetia, Sararanga), sedges (Gahnia); palms (many genera; especially young plants and Nipa and Sago palms), arum family (Colocasia, Alocasia, Xanthosoma, Dieffenbachia, Montrichardia and probably many other plants resembling taro and elephant's ears), bromeliads (the entire family Bromeliaceae including the edible pineapple), lily family in the broad sense (Dracaena, Cordyline, Collospermum, Astelia, Sansevieria, Smilax and probably many others), amaryllis family (Crinum), banana family (Musa, Strelitzia, Heliconia, Ravenala, Phenakospermum), ginger family (Zingiber, Curucuma), arrowroot family (Calathea, Maranta). In general only native plants will be found to contain native mosquitoes; introduced, cultivated or ornamental plants normally do not breed any mosquitoes but occasionally may breed introduced species.

The amount of water necessary for breeding is sometimes negligible and larvae have been found in leaf axils of native bananas where only a slimy muck could be recovered. In general, larvae in axils cling to the plant very closely and in some species are known to crawl from one axil to another. Considering this, great care should be taken in collecting and also in cleaning collecting tools, pipettes especially, between collections to prevent contamination.

Very little is known about the specificity of breeding sites on a single host plant but it appears that water and light conditions are frequently different in axils in different parts of a plant and it has been noted that different species may be present in upper and lower axils. Therefore an effort should be made to keep collections from different axils separate if this can possibly be done. The location of the breeding site with respect to the ground is also important. It should also be noted whether a plant is epiphytic or terrestrial and the height of the epiphytes above the ground noted. For tall terrestrial plants the height of the axil above ground should be indicated also.

The method of collecting must be adapted to the type of plant. Epiphytic plants, such as bromeliads, should be carefully dislodged from the tree and

lowered to the ground. All the extraneous debris is then carefully removed, washed and discarded and the leaves are cut to just above the level of the water and discarded. The plant is then carefully inverted into a clean bucket to remove the axillary water; some of this water is used to fill one or more plastic cups three-quarters full.. Each leaf base, starting from the bottom of the plant, is peeled off and washed in a pan of clean fresh water. The axillary water accumulating in the bucket and the wash water from the pan are then poured through a dip net bag which is then inverted and washed several times into the plastic cups to dislodge the immature stages and fine debris.

The same procedure may be followed for a terrestrial bromeliad by cutting off the plant at ground level or pulling it out carefully from the soil. If this is not possible, then the leaves are trimmed in place and a pipette is used to withdraw water from the axils into a container of suitable size from which one or more plastic cups are filled three-quarters full. Next, the upper (central) part of the plant is cut out a little at a time and the leaves washed in a pan of clean fresh water until the base is reached. Any water accumulating in the lower axils as this is being done should be pipetted out and placed with the original collection. The final concentration of the material is done the same way as above. The same technique is recommended for recovery of immature stages from terrestrial and epiphytic liliaceous plants as well as grasslike pandanus. A less laborious method in which the plant is not destroyed but which results in the loss of many larvae, is to withdraw the axillary water directly with a battery pipette into a plastic cup or some other suitable container. The axils should be washed repeatedly with fresh water.

With heliconias, young bananas and similar musaceous plants the flower stalks if present should be cut off first and examined for mosquitoes as indicated in the next section. The leaves are then all cut off carefully above the water line in each axil starting with the highest and discarded. If there is any visible water in the axils it is removed with a battery pipette and used to fill one or more plastic cups to the usual level. The plant is then cut off from the ground below the lowest axil containing water after all the soil around it is carefully scraped away. Next, place the whole plant into a large empty bucket without inverting and peel off each leaf starting from the base. One leaf at a time should be removed and washed in a pan of clean fresh water and examined carefully for larvae and pupae that may cling to it. The axillary water accumulating in the bucket is used to fill the plastic cups as needed; the rest after removal of large debris (be careful to wash it off) is passed through a dip net bag along with the wash water from the pan and the concentrate placed in the plastic cups in the usual manner.

With aroids and palms it is usually possible to collect immature stages without destroying the plant by withdrawing water from the axils directly with a battery pipette into a plastic cup or some other suitable container. Care should be taken to wash the axils repeatedly with fresh clean water. For some aroids, and palms as well, it may be necessary to use the longer method outlined for terrestrial bromeliads above.

For large, woody pandanus the leaves must be carefully cut above the water line and then the first method suggested for the aroids and palms used. Immature stages in pandanus lianas (Freycinetia) are readily collected directly with a battery pipette or even a smaller ordinary pipette but again it is essential to wash repeatedly with fresh clean water to recover all the immature stages.

Whenever possible the plants should be photographed and a record made of the flower size and color for identification purposes. Inflorescences (fresh or dead) as well as a specimen of leaf should be collected as herbarium specimens. Indicate on the collection form the name of the plant if known.

22. Flower bracts, spathes. Flowers or inflorescences with large horizontal bracts or spathes containing water provide breeding sites for a considerable number of species of mosquitoes of several genera. The majority of plants with flower breeding belong to the banana family (Strelitzia, Heliconia and Phenakospermum especially), gingers (Zingiber, Curcuma) and arrowroots (Calathea, Maranta) but others have been reported (Sapria in the Rafflesia family). To collect the immature stages, the inflorescence (flower stalk) is cut off and the bulk of the water is removed with a pipette of appropriate size to a plastic cup. The inflorescence is then placed in a pan of clean fresh water and each part is separated and carefully washed. The large debris is removed before the wash water is filtered through a dip net bag. The concentrate is then added to the water in the plastic cup in the usual manner. As in all other collections the material from each inflorescence is given a separate collection number. The name of the plant should be recorded in the blank space provided on the form and if possible a photograph taken. For specific identification of the plant inflorescences (fresh or dead) as well as a specimen of leaf should be collected as herbarium specimens. Several immature flowers should also be preserved in alcohol or formalin in a plastic vial.

23. Pitcher plants. The most specialized and unique breeding sites for mosquitoes are in the pitchers of carnivorous plants of the genera Sarracenia (New World) and Nepenthes (Old World). The latter has representative species of mosquitoes of nearly all the Old World genera which utilize container habitats and all of these (some doubtful exceptions) are restricted to Nepenthes. In the New World only 2 species of Wyeomyia are known from Sarracenia (eastern North America) but species of the related pitcher plant genus Heliamphora are known from the Guiana highlands (British Guiana and Venezuela) and may serve as a breeding site for other sabethines. Pitcher plants of the genera Darlingtonia (California and Oregon) and Cephalotus (southwestern Australia) apparently are not used by mosquitoes although they have not been thoroughly studied.

The immature stages in pitcher plants are not easily seen for the larvae are sluggish and remain in the bottom of the pitchers. To collect them the pitchers should be carefully detached, emptied of their contents and thoroughly washed with clean fresh water which should be added to the collection. Collections from different pitchers should be kept separate and given individual collection numbers (lots). Notation should be made of the species of plant and of the condition of the water. Care must be taken not to destroy whole colonies of pitcher plants, some of which are protected by law.

24. Traps. Immature stages of a number of species of container breeding mosquitoes may be obtained in bamboo pots, i.e. sections of bamboo internodes filled with fresh clean water and placed on the ground or suspended in suitable protected spots at different heights in clumps of bamboo or in trees. Species otherwise difficult or impossible to collect may be recovered in this way. Different types of bamboo traps should be used to simulate natural breeding sites, some completely open, others with small lateral openings, some cut from young green shoots of varying length, others from older woody culms. The water in the pots should be checked and periodically refilled to dislodge eggs laid above the water level. As suggested above in item 16, holes may be bored in live bamboo culms to produce artificial breeding sites.

Other types of traps, (such as wooden tubs of water set out in the forest), should be tried also, particularly during the dry season in areas where adults of the desired species are abundant. A notation should always be made of the type of trap used. Collections from individual traps should be kept separate and assigned individual numbers.

HEIGHT OF BREEDING SITE ABOVE GROUND. This should be indicated in meters for all collections in treeholes, bamboo, fallen trees, attached fruits, leaf axils and flower bracts, aerial pitcher plants and traps located above ground.

WATER. The condition of the water in every breeding site must be carefully determined and all appropriate items indicated by circling, underscoring or writing in.

1. Permanence. Permanent breeding sites have water present throughout the year; this can usually be determined by the presence of true aquatic plants in case of ground water habitats; crabholes, pitcher plants and the larger bromeliads and astelias usually contain permanent water. Semi-permanent breeding sites have water throughout most of the year but dry up by the end of the dry season for a month or longer; many swamps and the margins of lakes and ponds are semi-permanent; some container habitats, particularly large treeholes and the leaf axils of large bromeliads are semi-permanent. Temporary habitats are characterized by the presence of water for only short periods of time; most temporary ground pools are filled by rainwater but dry up quickly and lack aquatic and semi-aquatic vegetation; most container habitats (small treeholes, bamboo, leaf axils, etc.) have temporary rainwater.

2. Clarity. Indicate for all breeding sites whether the water is clear or turbid and if it is colored note the color.

3. Movement. For ground water sites indicate whether the water is stagnant or if it shows a slow, moderate or strong flow.

4. Salt content. Indicate for ground water sites if the water is fresh, brackish or salty.

5. Organic contamination. For all sites, particularly small containers, if the water is foul smelling, contains gelatinous or slimy material, or has fermenting organic material (e.g. decaying coconut meat) underscore the appropriate item.

VEGETATION IN THE BREEDING SITE. This section applies primarily to habitats on the ground. Check all the items and indicate the appropriate ones by circling or underscoring.

1. Abundance. Indicate the relative abundance or total absence of all types of vegetation and surface debris.

2. Small surface vegetation and debris. Indicate the presence of (1) flotage, i.e. floating sticks, chaff, leaves, (2) bacterial scum, (3) algae of all kinds.

3. Large vegetation. Indicate all types of conspicuous vegetation present in the site. Grassy vegetation includes all types of grasslike forms, herbaceous indicates forms such as cattails, sedges, rushes, etc. and woody, primarily shrubs and trees. For floating vegetation write in the name (water lettuce, water hyacinth, water lily; if known give the scientific name). Do the same for submerged vegetation (pond weed, filamentous algae, etc.).

BOTTOM. For all breeding sites indicate the presence and nature of the following materials in the bottom: 1. Inorganic material, whether it is mud, sand, gravel or rock. 2. Organic material: specify the type of decaying plant matter (leaves, sticks, fine sediment) and the type of decaying animal matter (feces, urine and domestic wastes).

INCIDENTAL COLLECTIONS

It may be possible for individuals not engaged specifically in a mosquito survey to make valuable collections of mosquitoes while in the field but without the time, equipment and facilities required for the type of collections mentioned in the preceding two chapters. The basic equipment necessary for such incidental collecting would be 2 or more killing tubes, an aspirator, a folding aerial net, a supply of plastic pillboxes with paper toweling or cellucotton, a medicine dropper, a medium sized pipette, a battery pipette, 2 or more plastic cups with lids, a supply of plastic bags, a supply of shell vials with 80% ethanol, absorbent cotton, entomological forceps, label strips and a supply of collection forms.

Wherever adults are encountered (for specific places see chapter on ADULT COLLECTIONS) they may be collected directly into killing tubes or gently blown from the aspirator into the killing tube after collection with the net or the aspirator. Mosquitoes should not be left in killing tubes for longer than 30 minutes and it is important that the tissue paper strips in the tubes be replaced as soon as they show evidence of moisture. After completion of a collection, fill out the collection form as indicated in the chapter on ADULT COLLECTIONS. Empty the killing tubes on a smooth clean surface in a place protected from strong air currents. Using the entomological forceps place the adults carefully in a plastic pillbox between layers of paper toweling or cellucotton (for details see chapter on KILLING AND PRESERVATION). Write out the collection number (lot) on a strip of paper and place the label in a separate layer above the specimens. Several collections may be preserved in one pillbox but be sure to mark these carefully. Pillboxes should be stored in a dry place and care must be taken that they are tightly closed. Use adhesive tape to seal them.

Wherever immature stages are encountered (see chapter on COLLECTION OF IMMATURE STAGES for specific sites) they may be captured directly with an ordinary pipette or a battery pipette or they may be dipped out of the water with a plastic cup by gradually lowering the cup around edges or by quickly scooping in more open situations. Transfer the immature stages with a pipette to a plastic cup with fresh clean water, serially through several cups if much sediment is carried over. Then kill the larvae and pupae by transferring them, one or a few at a time, to the shell vials containing 80% ethanol. To avoid diluting the alcohol excessively, use a medicine dropper and squeeze out most of the water while holding the dropper against a finger. Place no more than about 5-10 larvae or pupae into one vial depending upon their size. Insert a small loose plug of cotton into the vial above the immature stages (not touching) after they drop down to the bottom and place a label with the collection number above the cotton plug. Make sure that the immature stages are not trapped between the walls of the vial and the cotton. To speed up killing of immature stages place the vial, before and/or after transferring the larvae and pupae, into direct sunlight. Fill out the collection form carefully as indicated in the chapter on COLLECTION OF IMMATURE STAGES. Pay particular attention to collecting in container habitats (treeholes, crabholes, rockholes, plant axils and bracts, pitcher plants, plant parts on the ground and so on) for the largest number of species and the most interesting forms will be found there.

When dry treeholes that might serve as breeding sites are encountered, collect debris from the sides and bottom into plastic bags. Place a large label with the collection number inside the bag.

For storage and shipment of material follow the suggestions in the last chapter.

SORTING AND REARING

FACILITIES AND EQUIPMENT. A large cool room with electricity, running water and several tables or laboratory benches serves very well as a simple laboratory for mosquito rearing. The room should be ant-proof but if it is not, the legs of the tables or benches should be placed in cans of oil. Air-conditioning is a convenience for the workers but is not necessary or even desirable for mosquito rearing. Mosquito rearing may be done under much more primitive conditions, in a tent, camper, trailer, a passenger car and even on the trail, but in these cases the techniques and equipment described below must be modified to the particular situation. However the general technique is the same in all cases.

A laboratory or a collapsible stereoscopic field microscope is extremely useful but a good hand lens (10X to 20X) is usually sufficient. The basic equipment and supplies required for rearing and processing are (1) pipettes and medicine droppers, (2) aspirators, (3) dip net bags, (4) plastic, enamel or porcelain sorting bowls, (5) plastic cups with lids, (6) plastic cup cages, (7) plastic vials with lids, (8) plastic vial cages, (9) plastic pillboxes, (10) pencils, grease pencils, label strips, (11) paper towels and tissue, (12) forceps, camel's hair brushes. All the equipment should be for use exclusively in the laboratory and should not be taken into the field.

CARE OF COLLECTIONS AND SORTING. Immediately after a field trip all the collections must be checked carefully. If possible some sorting should also be done at this time but it may be postponed to a later hour or to the following day after taking care of emerged adults and the isolation of pupae and at least a few larvae (particularly if dealing with Psorophora). The grease pencil marks on all containers should be made plain if smudged.

1. Killed adults. The plastic cups and vials with killed adults should be opened and a drop or two of water added to the paper to keep the specimens from drying out. The processing of this material should be done within 24 hours of capture using the technique outlined under FIELD COLLECTED ADULTS in the next chapter.

2. Oviposition vials. The oviposition vials should be opened carefully and checked. If insufficient moisture is present a drop of water should be added. If the vial is excessively humid the female should be transferred to a fresh oviposition vial by loosening the lid of the old vial, carefully slipping the mouth of the new vial under this lid and allowing the female to move up into the new vial which is then capped and marked with the same number in grease pencil on the side and lid (the old lid may be used after it is dried). Set the vials aside for further processing as outlined below under PROGENY REARINGS.

3. Emergence and pupation. The plastic cups with immature stages require the greatest care. If a large number of adults have emerged in a cup, place an aerial net over it, carefully slip off the lid and let the adults move up into the inverted net, tapping on the side of the cup to drive them out. Now remove the net, confine the adults into the upper part of the net and remove them with an aspirator to a plastic cup cage marked with the collection number in grease pencil. If only a few adults have emerged they can usually be sucked up with an aspirator slipped in under the lid and then transferred to one or more plastic vial cages. The pupal skins are handled as indicated under EMERGENCE VIALS AND CAGES. The vial cages should be provided with a pencilled paper

label slipped under the lid. The cup and vial cages are set aside for 1 or 2 days after which the adults are killed and processed as indicated in the next chapter.

If there is a large number of pupae in any cup they should be transferred with a pipette to another cup with about 2 cm (about 1 inch) of fresh clean water and this cup covered with a cage top and the side marked with the collection number in grease pencil.

4. Sorting. The sorting of the immature stages and their separation for individual rearings, mass rearings and preservations can now be carried out. The sooner this is done the better and this should always be completed within 24 hours of capture. Work with one collection at a time going through the whole process before turning to the next collection. If more than one plastic cup contains immature stages from the same collection or if pupal and/or larval isolations were made in the field assemble them all together.

Generally, more than one species is found in a single collection of immature stages even when collections are made from individual breeding sites such as one crabhole, one treehole or one water-holding plant, but as a rule one species is dominant in a particular collection and the others are frequently represented by few specimens or by younger larval instars, by pupae or by eggs. It is more important to make fewer collections in which all the species have been recovered and the different stages of each (larva, pupa and adult, male and female) correctly associated (through individual rearings) than to make many collections in which these objectives have not been carried out. Therefore the emphasis below is on thoroughness of sorting and rearing but with indication of simpler, less time-consuming methods which may have to be followed at times for one reason or another.

When a collection contains little debris and sediment it is possible to do the sorting directly from the original plastic cup after the sediment has settled and as the larvae and pupae come to the surface.

First, all the pupae that were not isolated for rearing in the field are transferred one to a plastic vial in about 2 cm (somewhat less than one inch) of fresh clean water. The plastic vials are marked with the collection number in grease pencil. These will be later processed as indicated below under pupal rearings in the section on INDIVIDUAL REARINGS together with any pupae that may have been isolated from the same collection in the field. As a rule, all the pupae present in a collection should be isolated individually unless the collection consists primarily of pupae and the total number exceeds 15 in which case see under MASS REARINGS below for procedure to be followed. If it is obvious that several species are represented among the pupae or an unusual species is suspected a larger number should be isolated individually (see pupal rearings below).

Second, the fourth instar larvae are roughly sorted by eye to species, picked up one by one and placed into separate plastic cups for each species. Each cup is marked in grease pencil with the collection number followed by a dash and then a separate sublot number for each species, sublot 1 for one species, sublot 2 for the second species, and so on as necessary to sublot 9 for the ninth species. When more than 9 species are found in one collection assign another collection number, indicate that it is a continuation of the original collection number and use the 9 sublots in this new lot as needed. Use general appearance, color, size, movement, position in the water, size and shape of head capsule and antenna, hairiness of body, length and shape of siphon as criteria for distinguishing different species from one another. Frequently the pattern of abdominal pigmentation will help distinguish some

species, e.g. fourth abdominal segment unpigmented and contrasting with the other strongly pigmented segments. Leave the smaller instars in the original cup unless they can be associated with fourth instar larvae on the basis of the characters mentioned above.

Third, for each sublot isolate half of the total number of mature larvae, up to a maximum of 10, in plastic vials for individual rearings. When there is an uneven number of larvae the total number is treated as if it were the next even number for the purpose of making isolations, i.e. if only one larva is present it is used for an individual rearing, if there are 3, two are isolated, if there are 5, three are isolated and so on. The individual vials are filled with water from the original collection cup to a height of about 2 cm (somewhat less than one inch), marked with the collection number (lot) and the sublot number in grease pencil and set aside to be processed as indicated below under INDIVIDUAL REARINGS. The remaining larvae in each sublot, normally up to a maximum of 20, are set aside for killing and preservation (see next chapter) and any larvae left over in each sublot are transferred into a clean plastic cup with about 2 cm (inch or less) of water from the original collection, marked with the collection number (lot) and sublot in grease pencil and processed as indicated below under MASS REARINGS. If an unusual species is encountered and the number of larvae remaining in a sublot is greater than 50 the larvae should be divided equally for killing and for mass rearing. In case it is not practical to take care of a large number of mass rearings because of shortage of personnel or some other reason all the larvae remaining after isolation of individuals should be killed and preserved; NEVER DISCARD ANY MATERIAL ONCE COLLECTED.

The unsorted material left in the plastic cup(s) from the original collection, containing young larvae and marked with the collection number only should be treated as indicated below under MASS REARINGS.

Frequently it will be impossible to sort the larvae and pupae directly from the plastic cups brought in from the field because of the dark color of the water or the presence of a great deal of debris or sediment. In such cases the contents are agitated with a pipette and poured through a dip net bag. The original water is saved and returned to the original cup(s) or to clean plastic cup(s) plainly marked with the collection number (lot) in grease pencil. If the original plastic cup is discarded be sure to wash it off thoroughly to save any eggs that may be stranded on the sides and pass this water through the dip net bag. Now the concentrate in the dip net bag is dipped into the sorting pan(s) with ample quantity of clean fresh water and carefully washed off; if necessary use a stream from a squeeze bottle or a pipette to dislodge all the material. It may be necessary to subdivide the material for sorting into additional pans a little at a time. The procedure outlined above for the separation of pupae, sublots and individual rearings is now followed; only the original water is used for the larval rearing. When the sorting is completed, the water in the sorting pans, containing debris and small larvae, is passed through a dip net bag, the concentrate replaced into the original collection water in one or more plastic cups and the wash water discarded. Be sure to wash the sorting pans thoroughly and to add the concentrate to the plastic cups with the original water before they are further processed as indicated below under MASS REARINGS.

Great care should be taken to rinse thoroughly sorting pans and pipettes between processings of different collections to eliminate contamination.

It is sometimes impossible to go through all the steps indicated above for the sorting of material and to make lot and/or sublot mass rearings (see

MASS REARINGS below) in addition to individual rearings. It may be difficult even to sort the larvae to species. Larval isolations for individual rearings should always be made but in extreme cases the remainder of the collection may be killed and preserved as indicated in the next chapter.

WASHING CONTAINERS. All containers used for collecting, sorting and rearing must be washed thoroughly before they are used again. First, wipe off the grease pencil markings from the dry containers with a piece of absorbent cotton. Containers that are reasonably clean, without deposit of salts on the inner walls, may be merely rinsed several times in clean fresh water. Soiled plastic containers should be washed with water and ordinary soap only, never with detergents or with scouring or cleansing powders or compounds. Use a nylon test tube brush to remove stains and deposits. Be sure to wash out very thoroughly all the rubber bulbs after detaching them from the pipettes. Plastic vial cages should have the cotton replaced as soon as they show any growth of molds or become soiled (see GLOSSARY for method). Aspirators should also be cleaned periodically and the netting replaced on the plug.

ORGANIZATION OF LABORATORY. Even when a small number of collections is being reared it is important to organize all the material systematically in the laboratory to eliminate confusion and to save time. Two general methods are suggested below, the choice between the two depends on the amount of material, number of individual rearings and the available personnel.

When the number of collections is relatively small (less than 10) and the total number of individual rearings at any one time is less than 100, all the containers pertaining to each collection may be placed together, the general lot mass rearing, the sublot mass rearings, the individual pupal and larval rearings, the holding vial cages for adults, the collection record forms together with the label strips and the shell vials containing the larval and pupal skins. Small shallow boxes or special racks to hold the individual plastic vials and cages containing the various stages in groups are very useful but not essential. Under these conditions one shell vial may be used for both the larval and pupal skin of the same individual rearing. The larval skin is transferred from the rearing vial together with its label but the cotton plug is placed into the vial only after the corresponding pupal skin is later added when the larval label is withdrawn and matched with the pupal label; both labels are then placed above the cotton plug before final stoppering. It is important to have both labels in the vial for this provides a check for correct association of skins.

With a large number of collections (10 or over) and with more than a total of 100 individual rearings being carried out at one time, it is much more efficient to arrange the containers together in groups as follows: (1) all the mass rearings but with all sublots pertaining to one collection (lot) assembled in subgroups together with the general lot of the same collection, (2) all the individual vials with larvae, (3) all the individual vials with pupae, (4) all the cages (vial and cup) containing adults isolated for less than 24 hours, (5) the same as (4) but held over 24 hours. The collection record forms, with the strips of individual numbers attached, should be filed all in one place, where all the records will be made. With this arrangement each of the groups listed above is attended to one at a time until all the work for a group is completed and the records and labeling is done as indicated below under MASS REARINGS and INDIVIDUAL REARINGS. Much time is saved if the larval and pupal skins, each with its own identifying individual number, are placed in separate shell vials. The association of the stages is done later when the material is mounted.

It is very important to prepare for each collection strips of replicate numbers for labeling individual rearings as specified in the section on LABELING in the chapter on COLLECTION RECORDS. See also this chapter for labeling of material from sublots.

MASS REARINGS. Three types of mass rearings are distinguished here: (1) lot mass rearings, the general collection not sorted to species or the young larvae and eggs from the original collection that remain after the fourth instar larvae have been removed, (2) sublot mass rearings, the larvae sorted from the original collection, each presumably containing only one species and (3) progeny mass rearings. After sorting, a lot rearing with its sublot rearings are placed in a group in the mass rearing area and the cups covered by placing an inverted lid on top; it is not necessary or advisable to seal the container in the ordinary way. Mark on the outside of the cup in grease pencil the level of the water inside the cup. If marked evaporation takes place, the water lost should be replaced with distilled water if available, otherwise tap water will do.

Every cup must be examined twice each day. Examine one lot and its sublots at one time. If in the lot mass rearing an additional species is noted which has not been segregated as a sublot, sort it out, assign it a sublot and mark the plastic cup with the lot and sublot number in sequence following the last sublot already assigned in this lot. When the larvae in the new sublot(s) are mature, isolate some for individual rearings, set aside others for killing and preservation and retain the remainder for mass rearing (see CARE OF COLLECTIONS AND SORTING above for details). The lot mass rearings should be retained for at least 10 days to allow hatching and development of young larvae.

Check every lot and sublot in the collection record forms to determine whether further pupal or larval isolations are necessary. If the full quota of 15 pupal individual rearings has not been reached or if more rearings are desired in special cases, isolate additional pupae in individual plastic vials, mark the vials with the lot number in grease pencil and set the vials aside to be processed as indicated under pupal rearings in the section on INDIVIDUAL REARINGS below. If the full quota of 10 (more in special cases) larval individual rearings has not been reached, isolate in individual plastic vials full-grown fourth instar larvae. Label the vials with the lot and sublot number in grease pencil and set them aside to be processed as indicated under larval rearings in the section on INDIVIDUAL REARINGS below.

After the isolations are completed pick off all the pupae in each lot and sublot and place them in plastic vials with clean fresh water, up to 5-10 per vial (depending on size). If a great number of larvae pupate at once the pupae may be placed in a plastic cup cage (see GLOSSARY) for emergence, up to 200 per cup cage. Mark the vials or cups with the lot and sublot number in grease pencil, place them with the group of pupal material to be treated later as indicated below under EMERGENCE VIALS.

Now fill plastic vials about three-quarters full with 80% ethanol, cover them with lids, mark them with the lot and sublot number on the side and on the lid and place a paper label with similar data inside each of the vials. One vial is prepared for each lot or sublot mass rearing and placed next to each of them until the mass rearings are completed. In each lot and sublot mass rearing all the larval skins and dead larvae and pupae should be picked up with a pipette and transferred to a plastic bowl of clean fresh water; this will serve to wash debris from the specimens. From the bowl transfer all the skins and all

firm larvae and pupae to the proper vial with ethanol; discard all other material. After the mass rearings are completed the preserved material will be processed as indicated in the next chapter (INCOMPLETE REARINGS).

In general, it is not necessary to add food to the mass rearings if ample debris was present in the original collection. However if development is slow a small amount of finely ground dog biscuit or laboratory chow may be added from time to time. For anopheline larvae this should be sprinkled lightly on the surface. For all other larvae except carnivorous forms a slurry should be made first by adding a little water to the ground food and mixing it thoroughly. A drop or two of the slurry should be placed with a pipette into the bottom of the plastic cups.

Carnivorous larvae present a special problem in mass rearings. As soon as discovered they should be isolated in individual vials or cups and each larva provided with several small larvae of some readily available species (see section on SPECIAL REARINGS below).

INDIVIDUAL REARINGS. Individual rearings are made from either pupae (pupal rearings) or larvae (larval rearings) which have been isolated in individual plastic vials either in the field or in the laboratory and marked with the collection (lot) number and the sublot. Each vial must be provided now with an individual rearing number which will identify the stages of each individual throughout the rearing and processing (see LABELING in the chapter on COLLECTION RECORDS).

Pupal Rearings. Assemble all the vials of pupae marked with a given collection number (lot) and have the collection record form for that collection at hand. Mark an entire strip of duplicate numbers in the -100 series with the collection number in front of the dash. Cut off a duplicate set of numbers from the top of the strip and insert this label on the outside of the vial between the lip of the vial and the lid. Continue until all the pupal isolations are labeled. On the collection forms place a check mark in front of all the numbers that have been assigned and attach with a paperclip the remainder of the label strip to the collection form. This will be used to mark any additional pupal isolations that may be made from the mass rearing of that collection. After labeling, all the pupal rearing vials are placed together and processed further as indicated in the section on EMERGENCE VIALS below.

Larval Rearings. Assemble all the vials of larvae (or larval skins and associated pupae) marked with the same lot number and arrange them in groups according to sublots. Have the collection record form for that collection (lot) at hand. Mark full strips of triplicate numbers in the -10, -20, -30 series as needed for each of the sublots by writing in the collection number (lot) in front of the dash. Working with one sublot at a time cut off a triplicate set of numbers from the top of a strip and insert this label on the outside of the vial between the lip of the vial and the lid. Continue until all the larval isolations are labeled for a given sublot. On the collection form place a check mark in front of all the numbers that have been assigned and attach with a paperclip the remainder of the label strip to the collection form. Do the same for all the sublots in the same lot and then go on to the other lots and repeat. The label strips attached to the collection form will be used to mark any additional larval isolations that may be made in the future from the sublot mass rearings. After labeling, all the larval rearing vials are placed together and processed further as indicated in the section immediately below.

PUPATION VIALS. Vials containing isolated larvae, marked with individual rearing numbers (see Larval Rearings above), should be examined twice a day for pupation preferably in late forenoon and late afternoon. Check through all the vials and set aside all those containing larval skins before further processing. Pour the contents of such a vial into a small clean sorting bowl with fresh clean water. Transfer the pupa with a pipette into a clean plastic vial containing 2 cm of clean fresh water; cut off two of the triplicate numbers from the label and insert this label on the outside between the lip of the vial and the lid. This marked vial is now placed with the emergence vials and processed as indicated in the section below. Next, rinse out the original isolation vial with clean water and fill it with about 5 ml of 80% ethanol. Transfer the larval skin with a pipette into the vial with alcohol and attach the remaining number of the triplicate set in the usual manner. This marked vial is now set aside to be processed as indicated in the section on SKINS in the chapter on KILLING AND PRESERVATION. This entire process is repeated for all the larval isolations.

All vials containing dead or moribund larvae should be set aside to be promptly processed after completion of the care of the individual rearings as indicated in the section on PARTIAL REARINGS in the next chapter. DO NOT DISCARD THEM OR REMOVE THEIR LABELS.

If only a small number of collections is being processed in the laboratory (see CARE OF COLLECTIONS AND SORTING above) the larval skins can be put into individual shell vials filled with 80% ethanol, each with its identifying label, at this time. For directions see again the section on SKINS in the following chapter.

No food should normally be added to pupation vials except for carnivorous forms. For these as well as for Mansonia and other larvae requiring special attention see the section on SPECIAL REARINGS below.

EMERGENCE VIALS AND CAGES. Vials containing isolated pupae, marked with individual numbers, either from direct field collections or from mass lot rearings and individual larval rearings should be examined twice a day, preferably in early morning and late afternoon. Check all the vials and set aside all those with emerged or drowned adults for processing. To remove the viable emerged adult loosen the lid and carefully slip in and replace it by the mouth of an inverted plastic holding vial (see GLOSSARY). Tap on the side of the pupal container to induce the mosquito to enter the holding vial and quickly stopper it with the original lid. Cut the duplicate identifying number into 2 strips, attach one of these under the lid in the usual manner. This vial will now be processed as indicated below under HOLDING VIALS AND CAGES. Next pour some 80% ethanol (about equal in volume to the pupal water) into the vial containing the pupal skin and drop in the other label. Put this vial aside to be processed as indicated in the section on SKINS in the next chapter. The entire process is repeated for all the pupal vials.

All vials containing dead or moribund pupae and drowned, partially emerged or very weak adults stuck to the water film should be set aside and processed prompty after completion of the care of individual rearings as indicated in the section on PARTIAL REARINGS in the next chapter. NEVER DISCARD THIS TYPE OF MATERIAL OR REMOVE LABELS FROM IT.

When plastic cups with screen tops are used for the mass emergence of adults they should be checked twice a day as in the case of emergence vials. For each collection all emerged adults are first picked up with an aspirator inserted through the slit in the top and transferred to a similar cup without

any water. This plastic cup cage is marked with the lot and sublot number as required and placed with the individual holding vials to be treated as indicated below. If only a few adults emerge at one time they can be transferred to small emergence vials similar to those used for individual rearings, up to 5 per vial. After the adults are removed all the pupal skins and dead pupae in the plastic cup are transferred with a pipette to a plastic vial filled with 80% ethanol. Excess water in the pipette should be removed before dropping the mosquitoes into the vial. Mark the vial with the lot and sublot number as required and keep the vial by the plastic cup with the live pupae until emergence is completed. The vial will then be processed as indicated in the paragraph on Mass Rearings in the section on REARED ADULTS in the next chapter.

HOLDING VIALS AND CAGES. Reared adults should be kept for at least 24 hours, preferably for 48 hours, before being killed and processed as indicated in the section on REARED ADULTS in the next chapter. If they are kept for 24 hours there will be 2 groups of cages at any one time, one group consisting of those being isolated on a given day, another those isolated on the day before. If adults are held for 48 hours there will be a third group. Mark each group plainly with the date the adults were isolated.

The individual holding vials require relatively little attention; it is not necessary to feed or to open the cages during the holding period. The vials must always be tightly stoppered. Check at least once a day through all groups, process dead specimens immediately or as soon as possible, following the directions in the section on REARED ADULTS in the next chapter. After the required 24- or 48-hour holding period process the specimens in that group following the same directions.

The plastic cup cages should have a small wad of moistened absorbent cotton resting on top of the netting which is covered loosely with an inverted lid. Remove all dead adults as soon as noted with an aspirator inserted into the slit in the netting; the cotton plug must be carefully replaced. Place these adults into a clean dry plastic vial together with the required label and process this as soon as possible following directions in the section on REARED ADULTS in the next chapter.

Plastic vials containing several adults should be handled the same way as the plastic cup cages.

PROGENY REARINGS. Female mosquitoes isolated in oviposition vials (see the chapter on the COLLECTION OF ADULTS and the section on CARE OF COLLECTIONS AND SORTING above) require considerable attention in the laboratory. First replace the solid lid with fine nylon netting held on the vial by another plastic lid whose center has been cut out. Place a very small wad of cotton moistened (not dripping) with 10% sugar solution on top of the netting and cover loosely with the original solid plastic lid. The oviposition cages should be checked daily and the sugar wad renewed if any mold develops or if it dries out. It is suggested that a blood meal be offered daily to each female by placing the screened top against the inner part of the forearm or finger. If there is any danger of disease transmission or if the female does not take human blood various mammals or birds should be tried as a source of blood by placing the screened top of the vial on a suitable area of a confined donor. It is very important to provide a daily blood meal to certain species in order to obtain oviposition, particularly in the genus Psorophora.

The place of oviposition in nature varies with the different genera (sometimes species within a genus) and it is therefore necessary to provide conditions suitable for the particular species at hand. For Anopheles a few drops

of water should be added through the screen top to maintain a thin film over the cotton in the bottom of the vial by applying the tip of a medicine dropper to the netting and forcing the appropriate amount of water through it; avoid drenching the mosquito. For Culex, Aedeomyia, Uranotaenia, Culiseta, Mansonia, Toxorhynchites and other forms generally laying eggs on the water surface, singly or in rafts, more water should be added so that it stands about 1 cm above the level of the cotton. For many Mansonia an additional requirement is a small disc of wet-strength paper placed on the surface of the water (see SPECIAL REARINGS). For all other groups, including particularly Aedes, Haemagogus, Psorophora and related genera, there should be little or no free water on the cotton on the bottom of the vial so that the strip of paper towel on the inside of the vial will show a gradient of humidity. Eggs will be laid by these forms primarily on the strip of paper toweling. For other genera, variations of these methods should be tried to simulate the conditions in the normal oviposition sites. Paper toweling of varied texture, color and absorbency as well as strips of wet-strength paper should be tried. It may be necessary to place the oviposition cage in the dark for some species or to wrap dark paper completely or partially around the vial.

Gravid females collected in the field will generally oviposit within 3 to 4 days if they will lay eggs at all. It is usually not necessary and may be even detrimental to offer blood to gravid females. Females that are blooded in the field and laboratory will generally take from 5 to 10 days or even longer before ovipositing and these should be offered a blood meal daily as indicated in the first paragraph of this section.

The daily routine of care for the oviposition vials should be as follows, preferably carried out early in the morning: (1) checking and maintaining the proper level of water and moisture in the vial; if moisture condenses on inner walls remove the solid plastic lid from the screen top; if molds develop on the cotton or elsewhere change to a new vial, (2) moistening or replacing the sugar wad, (3) offering a blood meal (this may have to be done at night or in the dark for some species), (4) checking thoroughly for eggs and (5) processing the parent female and her clutch of eggs.

If a female dies before laying eggs, examine her carefully and if it appears that she may contain fully developed eggs dissect the abdomen. If eggs with fully formed plump egg shells are found treat the remains of the parent female and the eggs as indicated in the next paragraphs. All dead nongravid females should be saved in the original oviposition cages and processed as soon as possible as indicated in the next chapter under FIELD-COLLECTED ADULTS.

Some gravid females will refuse to lay eggs. In such instances forced oviposition should be tried. Cutting off the wings under light anesthesia and allowing the female to recover on moist paper toweling in the oviposition cage may induce oviposition. Decapitation of the female is a more drastic stimulus but works well with some species. Simple CO_2 anesthesia may also work and should be tried using a cork ejector of the type described by Bruce-Chwatt (1964).

Each cage containing a parent female and her eggs should be processed promptly. Up to this time these cages have carried only the collection number (lot). Now each female and her progeny is assigned a sublot number within the original lot, from -1 to -9. Very seldom will there be more than 9 progeny rearings from a single lot; if this happens, a new lot number is assigned for the continuation of the original one, making available sublot numbers for 9 additional progenies.

Before anything else, record on the front of the proper collection form in the SUBLOTS section the assignment of a sublot number by placing a check mark in front of the number assigned. Next prepare two paper labels showing the lot and sublot numbers; to one of these add the letter P following the sublot number, this label will identify the parent female, the other, replicated as needed, her progeny through the mass rearings. Now, the parent female is removed in the usual way to a clean plastic vial which is capped with a solid lid under which the identifying label has been inserted. The vial is set aside to be processed later as indicated in the next chapter in the section on REARED ADULTS.

The eggs and mass rearings obtained from the ovipositing females are handled in different ways depending on the genus:

(1) For Anopheles, a ring of wax paper is placed on the surface of the tap water in a plastic cup (about three-quarters full), which is marked with the lot and sublot number. The eggs are transferred as soon as possible to the rearing container by removing the paper strip and cotton from the oviposition cage and immersing them in the water through the center of the wax ring. Eggs remaining on the sides of the oviposition vial are picked up gently with a camel's hair brush and dipped into the water in the center of the ring. Any eggs stranded on the walls of the plastic cup or floating on the outside of the wax ring are transferred with a camel's hair brush to the water surface inside the ring. About 10 eggs from each clutch should be preserved following the instructions in the section on EGGS in the following chapter. The mass rearing is now set aside. When the first larva hatches (in about 3-5 days) a very small piece of yeast cake is placed on the inside rim on the wax ring so that it touches the water. When all the first instar larvae have hatched the wax ring is removed. Finely ground dry food is sprinkled very sparingly twice a day on the water surface. For further treatment see the general instructions for mass rearing, preservation of larvae and isolation of individual rearings below.

(2) For Culex and other forms whose eggs are laid in masses or individually on the water surface, transfer the eggs with a camel's hair brush into a plastic cup three-quarters filled with tap water. Label the cup with the lot and sublot number in grease pencil. When the first larvae appear place a small amount of food slurry in the bottom of the plastic cup once or twice a day as needed.

(3) For species of Mansonia transfer the egg mass or the disc of wet-strength paper to which the eggs are attached, to a plastic cup three-quarters full of tap water. Label the cup and feed the larvae as indicated above. Place additional discs or strips of wet-strength paper on the water surface as needed for the attachment of larvae. See the section on SPECIAL REARINGS below for further processing.

(4) For Aedes, Psorophora, Haemagogus and other forms whose eggs are laid primarily above the water surface the following procedure should be followed: (a) Initial period of conditioning: the oviposition vials should be capped with solid lids to provide a humid environment for the development of the eggs. After 3 or 4 days examine 1 or 2 eggs under a microscope or with a hand lens. If the eggs are still plump they are probably viable and ready for drying. (b) Period of drying: replace the solid lid with a screen top and allow the eggs to dry. The period of drying will vary with different species and has to be determined empirically. Examine 1 or 2 eggs periodically to determine whether they remain plump (viable) or shrivel (dead); if they shrivel it probably means that in this particular species the period of drying should be omitted. (c) Period of flooding: if the eggs remain plump after drying for 3-5 days, fill the

vial with tap water or preferably rainwater and empty all the eggs into a plastic cup marked with the lot and sublot number. Fill the plastic cup about three-quarters full of water and add 1 tablet of vitamin C, a drop of food slurry and a very small piece of yeast cake. If the eggs are viable and properly conditioned hatching should occur within 30 minutes. If they fail to hatch transfer them to a dry strip of paper toweling placed in the original oviposition vial, cap the vial with a screen top, allow the eggs to dry for several days and repeat the flooding one or more times until hatching takes place.

For all progeny rearings a sample of 5-10 eggs should be preserved whenever possible before mass rearing is begun. See the section on EGGS in the next chapter for methods of preservation. Whenever possible a sample of 5-10 specimens of each instar (including the pupa) should be preserved following the directions in the section on WHOLE LARVAE AND PUPAE in the next chapter. Wait until nearly all the individuals have reached a given instar before selecting specimens of that instar for preservation to avoid a very uneven sex ratio in the final rearing (males develop faster).

Set aside all the progeny rearings as a subgroup with the general mass rearings and treat them as outlined above in the section on MASS REARINGS, preserving all the cast skins and marking all the material with the appropriate lot and sublot numbers and processing it as outlined in the next chapter. Be sure to feed the larvae regularly but not excessively. When all the individuals in a given progeny rearing are in the fourth instar, isolate 10 specimens in plastic vials for individual rearings. Assign each specimen an individual rearing number in the usual manner using the label strips with triplicate numbers. For example, the individual rearings from the progeny mass rearing 52-1 will be identified as 52-10, 52-11, 52-12 and so on to 52-19; those from progeny mass rearing 52-2 will be identified as 52-20, 52-21 and so on to 52-29. Attach the labels under the vial lid in the usual manner. On the back of the collection form place a check mark in front of all the numbers that have been assigned. The individual vials are now placed and processed together with all the others as indicated in the section on INDIVIDUAL REARINGS above.

Progeny rearings are very time-consuming and require a great deal of attention. Therefore with limited personnel only a few can be carried out at any one time and some of the steps may have to be eliminated, e.g. individual isolations and preservation of samples of all stages. However, progeny rearings are extremely important for taxonomic studies and every effort should be made to carry out the full procedure as outlined above for at least one female of each species.

EGG REARINGS. Egg rafts or individual eggs collected in the field for rearing should be processed, in different ways depending on the type of egg, according to the methods suggested in the section on PROGENY REARINGS above. Thereafter the rearings should be handled as indicated in the section on MASS REARINGS except that food must be added regularly. If more than one species is noted, sublots should be made. When all the fourth instar larvae are nearly mature proceed as indicated in the section on CARE OF COLLECTIONS AND SORTING to separate larvae for preservation and for individual rearings.

REARINGS FROM DRY MATERIAL. Samples of soil from depressions or edges of temporary ground pools and debris from treeholes and other container habitats (see section on COLLECTING EGGS AND DRY MATERIAL in the chapter on COLLECTION OF IMMATURE STAGES) often contain mosquito eggs that will hatch upon flooding. Place material from such a collection

in a plastic cup marked with the collection number, fill the cup about three-quarters full with rainwater or tap water and add a tablet of vitamin C. Some larvae should hatch within 30 minutes but others may not appear for about a day. If no hatching at all takes place concentrate the material with a dip net bag and dry the concentrate on paper toweling in a plastic cup. The process may have to be repeated more than once to induce hatching in some species.

Once larvae hatch the rearing should be handled as indicated above in the section on MASS REARINGS and separation of sublots and isolation of individual rearings as indicated in the section on CARE OF COLLECTIONS AND SORTING.

SPECIAL REARINGS. Some species require special attention and/or methods in individual and/or mass rearings. Only two specific cases are mentioned below but if difficulty is encountered with other types of larvae, variations in rearing techniques should be tried to reproduce conditions analogous to those in the natural habitat of the species in question.

Immature stages of the genus Mansonia may be reared using the simple technique described by Laurence, Page and Smith (1962). Discs of wet-strength paper, 1 cm in diameter, are placed on the water surface of the rearing container, 1 disc to an individual rearing, several for mass rearings. If this special type of paper is not available, use tough commercial grade paper towels. The discs may have to be replaced if the larva becomes detached. Pupation takes place on the underside of the disc with the pupa attaching itself firmly to the paper. To transfer the pupa lift the disc with forceps and place it on top of the water in the emergence vial.

Carnivorous larvae are usually readily recognized by a large head which is strongly produced in front of the antennae or by very strongly developed and projecting maxillae or mandibles. Most carnivorous forms are generally very sluggish but show sudden jerky movements when alarmed or when attacking their prey. As soon as noted, carnivorous larvae should be isolated in individual containers and provided with several small mosquito larvae of some common species. Development of these larvae will be rapid if the supply of prey is maintained. At pupation be careful that the correct larval skin and pupa is preserved for the larval vial may contain several cast larval skins and several pupae of the prey species.

KILLING AND PRESERVATION

EQUIPMENT AND SUPPLIES. The following equipment and supplies are needed for killing and preserving mosquitoes: (1) killing tubes, (2) aspirators, (3) aerial net, (4) ordinary and entomological forceps, (5) large and small plastic pillboxes, (6) paper toweling or cellucotton cut in squares to fit the pillboxes, (7) absorbent cotton, (8) gelatin capsules, (9) label strips, (10) 80% ethanol, (11) 5% and 10% formalin (2% and 4% formaldehyde respectively), (12) shell vials with neoprene stoppers, (13) small pipettes and medicine droppers, (14) sorting bowl, (15) dissecting needle, (16) beaker or pan for hot water.

FIELD-COLLECTED ADULTS. Adult mosquitoes collected in the field are usually killed immediately upon capture and placed in plastic cups or vials with paper toweling or tissue paper (see chapter on COLLECTION OF ADULTS) to be processed in the laboratory. Specimens obtained in incidental collections (see chapter) have to be processed in the same manner as outlined below, but

usually in the field. It is not advisable to make final mounts of adult mosquitoes on pins or points during a survey because of the time involved and because it is difficult to take proper care of mounted specimens and to ship them. If, however, final mounts have to be made, it is suggested that adults be glued with "Ambroid" cement on the right side, legs facing the pin, on heavy paper "points" on #3 insect pins with nylon heads.

The most practical method of handling field-collected adults is to preserve and store them in pillboxes for shipping and future mounting (for exceptions see ADULTS IN ALCOHOL). Many different types of pillboxes have been used quite satisfactorily but it is recommended that for the sake of uniformity plastic pillboxes be used. Cellucotton or household paper toweling cut to fit exactly into the pillbox should be used in preference to absorbent cotton to hold and protect the mosquitoes within the pillbox. With plastic pillboxes it is usually not necessary to use a mold preventative but if, for some personal reason, one must be used it is suggested that only 1 or 2 tiny crystals of thymol be anchored with glue to the bottom of the pillbox. NEVER PLACE LOOSE CRYSTALS OR FLAKES OF ANY KIND in a pillbox because of the danger of injury to the mosquitoes. If difficulty is encountered with specimens being drawn against the sides of the plastic pillbox, see STATIC ELECTRICITY in the GLOSSARY.

Place a cut sheet of paper toweling in the bottom of the pillbox. Using entomological forceps remove the mosquitoes from the plastic cup or vial to a sheet of paper before transferring them to the pillbox. Carefully arrange on top of the paper toweling in the pillbox a single layer of mosquitoes with legs stretched out and wings folded over the abdomen, the specimens spaced so that they do not touch each other. Never dump a mass of tangled specimens into a pillbox. Place another cut sheet of paper toweling on top of the layer of specimens and arrange another layer of mosquitoes. Repeat the process until all the mosquitoes from a collection have been arranged or until the last layer of mosquitoes comes up to the level of the shoulder of the pillbox. Place another cut sheet of paper toweling over the last layer of specimens and on top of that a wisp of of fluffed out absorbent cotton extending to all sides and corners of the pillbox and projecting very slightly over the top of the pillbox so that when the lid of the pillbox is put on, the cotton will barely touch it. Place the label, face up, upon the cotton in the center of the pillbox and close the box with the lid. Under no circumstance should any significant pressure be used in placing the specimens, the paper toweling or the cotton; this would result in flattening the specimens. The paper layers and cotton should rest lightly to barely hold the specimens and prevent them from moving. There is no need to seal the pillbox with tape in the laboratory but it is essential to do so when the processing is done in the field where the pillboxes are subjected to rough handling.

Use as many pillboxes as needed for a given collection and label each pillbox carefully with the collection number. It is not advisable to put more than one collection (lot) into one pillbox but if this has to be done because of many small collections, place a label on a sheet of paper toweling over the specimens from one collection and on top of the label, place another sheet of paper toweling on which specimens of the next collection will be arranged.

Pillboxes must always be stored in a dry, pest-free place. See the next chapter for further directions.

ADULTS IN ALCOHOL. With some soft-bodied mosquitoes, primarily dixa-midges, preservation in 80% ethanol is preferable to storage in pillboxes. After killing in the usual manner, specimens from field collections, mass

rearings, or individual rearings are simply dropped one by one, head first, into a shell vial filled with the alcohol. Separate vials are used for each individual rearing, each sublot and each lot. Do not place more than 10 specimens in one shell vial. A loose cotton plug is inserted into the vial and pushed down to just above the level of the specimens. The label is placed above the plug and the shell vial is capped with the neoprene stopper in the usual way. A record of the material should be made on the collection form as indicated at the end of the section on REARED ADULTS.

Poorly hardened, moribund or drowned specimens from mass rearings should also be preserved in alcohol.

REARED ADULTS. All reared adults after being held for 24 to 48 hours in plastic vials or cup cages (see HOLDING VIALS AND CAGES in preceding chapter) should be killed and processed as indicated below. Parent females in PROGENY REARINGS should be processed immediately after oviposition following the procedure for individual rearings below.

Individual rearings. Process all the adults from individual rearings (marked with lot number and 2 or 3 digit individual number) that have been held for the length of time (24 or 48 hour period) together at some convenient time in the afternoon or evening. To speed the process have at hand 2-5 killing tubes to be used serially. Loosen the lid of a holding vial and holding the vial upside down slip its mouth over that of a killing tube. The adult will be knocked down by the chloroform within seconds and will fall into the killing tube. To hasten the knockdown tap on the vial. Remove the vial and hold a thumb over the mouth of the killing tube until the specimen ceases struggling. Place the label inside the killing tube, stopper the tube and set it aside. Proceed in the same manner with 1-4 other adults and place the killing tubes in sequence. After all the tubes are used return to the first one for the second step in processing as indicated in the next paragraph. After this step is finished kill another adult with the emptied tube and place it in sequence after the others. Continue in this manner until all the adults are processed. Time the processing to allow the adult to remain in a tube at least 5 minutes but not longer than 10 by using the appropriate number of killing tubes.

Transfer the adult and its label from the killing tube to a clean white card. Pick up the adult with entomological forceps by the legs and let it fall head first into the larger half of a gelatin capsule. If the mosquito fits snuggly, tap the bottom of the capsule. Fluff out a very small piece of absorbent cotton and smooth it out on one end. Introduce the smooth end into the gelatin capsule to keep the mosquito from moving. The cotton must not touch the specimen and should not project outside the capsule half. Next place the label on the outside of the capsule half containing the specimen so that one end of the label extends just beyond the upper margin of the capsule and slip the smaller half of the gelatin capsule over it and the larger half as far as it will go.

A small plastic pillbox should be used to store the adult if it is too large to fit into a gelatin capsule or if gelatin capsules cannot be used at all because of excessive humidity. Place a square of paper toweling cut to fit exactly inside the pillbox on the bottom of the box and transfer the specimen to it. If difficulty is encountered with specimens being drawn against the sides of the plastic pillbox, follow the procedure under STATIC ELECTRICITY in the GLOSSARY. Next place loosely another square of paper toweling over the mosquito. Fluff out a small piece of absorbent cotton and use it to fill the plastic pillbox very loosely; this should be a mere puff of cotton fibers. Never stuff the pillbox

with a wad of cotton or exert any pressure. Next place the label on top of the cotton and cover with the pillbox lid. The bottom square of paper toweling may be left out so that the specimen is visible on the bottom of the pillbox but the mosquito should always be protected from the cotton by the upper square of paper.

After all the specimens have been processed a record of them must be made on the back of the collection forms. Place a check mark in pencil in the appropriate column in the line for the corresponding number for either a male (M) or female (F). If the sex is not readily determined place the check mark on the line between the two columns. If an adult escapes during processing make every effort to recover it. If there is any doubt at all that the specimen recaptured is the one that has escaped indicate this under REMARKS. If it is lost place 0 on the line between M and F.

Shell vials of the same type as used for storage of alcohol preserved material or other small shell vials of moderate diameter (up to 12 mm) may be used instead of gelatin capsules or small pillboxes. Let the mosquito fall into the bottom of the vial head first, insert a fluffed out piece of absorbent cotton to just above the legs of the mosquito, place the label on top of the cotton and cap the vial with the neoprene stopper.

Gelatin capsules are water soluble and great care must be taken not to get them wet. They must be stored in a dry and pest-free place. See the next chapter for further directions for the storage and shipment of gelatin capsules, pillboxes and shell vials.

Mass Rearings. Process at one time all the adults from mass rearings (marked with lot number or a lot number and a sublot number of 1 digit) that have been held for the same length of time (24 or 48 hour period). If the specimens are in vials use killing tubes as indicated for individual rearings above. Be careful with vials containing more than one adult. It may be necessary with these to release the specimens in a net first and then recover them with an aspirator or a killing tube. For specimens in plastic cup cages remove the adults one or a few at a time with an aspirator inserted through the slit in the screen top and blow them out gently into killing tubes.

Process all the adults from one lot or one sublot together, making sure that labels are placed in all the killing tubes. Make additional labels as needed. After the specimens have been in killing tubes for 5-10 minutes place them in large pillboxes using the procedure outlined above under FIELD-COLLECTED ADULTS. If there is only one specimen in a given lot or sublot it may be placed in a small pillbox using the technique described under Individual Rearings above.

After all the specimens have been processed a record of them must be made in the section on SUBLOTS on the front of the collection forms. Place a check mark in the appropriate column in the line corresponding to the sublot number for either or both male (M) or female (F). If the sex cannot be readily determined place the check mark on the line between the two columns (as also indicated under Individual Rearings). For collections not subdivided into sublots use the line for sublot -1. Store and pack the material as suggested in the next chapter.

WHOLE LARVAE AND PUPAE. It is essential to preserve an adequate sample of whole larvae of every species from every field collection and from all progeny rearings (see directions for material to be preserved in the preceding chapter on CARE OF COLLECTIONS AND SORTING, PROGENY REARINGS, EGG REARINGS and REARINGS FROM DRY MATERIAL). It is

also desirable to preserve some whole pupae if the material is available and time permits. To be useful for taxonomic purposes, whole larvae and pupae must be killed and preserved carefully so that the body shape and all structures, particularly hairs, are retained. Although it is possible to obtain fairly satisfactory material by killing larvae and pupae directly in the alcohol in which they will be preserved, this method is not dependable and should be used only where facilities or time for the technique outlined below are not available.

The larvae and pupae set aside for killing and preservation in a plastic cup marked with the lot and sublot number are first transferred with a pipette to a sorting bowl with fresh clean water as a washing procedure. With a small number of larvae the following procedure may be followed. If much debris or sediment is still present, additional serial transfers should be made until it is eliminated. Next a beaker or pan of water is heated to about 60 C (140 F) and the larvae are dropped into it one at a time with a minimum of the water from the sorting bowl. As soon as the larvae float up to the surface they are transferred with a pipette individually with a minimum of water to a container of 80% ethanol. After 5 minutes they are again transferred with a pipette in the same manner to a plastic vial, or a plastic pillbox with 80% ethanol. The size of this container will depend on the number of larvae; it should be big enough to accommodate all the larvae in a single layer on the bottom. Prepare a paper label in pencil, place it inside the container and cap the container tightly. The larvae should be allowed to harden for at least overnight before transfer to shell vials.

If there are more than 20 immatures to be killed from one collection, they should be washed, as above, and then they may be poured onto a small screen and this screen dipped into the container of hot water until the larvae are killed. The screen, with the larvae still in it, is then inverted and washed off in the first container of 80% ethanol from which they are transferred with a pipette into a plastic pillbox with 80% ethanol for hardening.

After the hardening period (overnight or longer) the ethanol in the container is pipetted off and replaced with fresh 80% ethanol. Next the larvae are transferred with a pipette to a shell vial filled with 80% ethanol. No more than 20 medium-sized larvae (such as C. quinquefasciatus) should be placed in one shell vial. If the larvae do not sink down immediately, tap the sides of the shell vial with a pencil. Next insert a plug of cotton into the shell vial forcing it down to a level just above the larvae. The plug should have a smooth bottom and should fit easily into the vial; never use a tight wad of cotton for a plug. On top of the plug place the label and bring the level of the alcohol to near the top of the vial. Put on the neoprene stopper and release the pressure by inserting a needle between it and the inner wall of the shell vial.

After the material is processed a record should be made on the front of the collection forms in the section on SUBLOTS. Place a check mark in the L (whole larvae) and/or P (whole pupae) column on the line corresponding to the sublot number; if only a lot number is found on the label use the line for sublot -1. Store and pack the material as suggested in the next chapter.

SKINS. The most valuable material for taxonomic purposes are the associated larval and pupal skins from individual rearings and the corresponding adults. The greatest care must be taken in processing these. When many mass or progeny rearings are carried on at one time no attempt should be made to preserve the corresponding larval and pupal skins in one vial. However with only a few rearings, the skins may be associated in one vial as indicated in the section on PUPATION VIALS in the preceding chapter.

The larval skins and the pupal skins in individual plastic vials containing dilute alcohol that have been set aside for preservation (section on PUPATION VIALS and EMERGENCE VIALS AND CAGES in the preceding chapter) must be processed the same day, preferably immediately after completion of the work on individual rearings. Empty the contents of a vial into a plastic pillbox with 80% ethanol resting on a white surface; put the label into the pillbox also. With a pipette transfer the skin into a shell vial filled with 80% ethanol. If the skin does not sink, place a finger on the mouth of the shell vial and invert the shell vial several times until the skin is wet and sinks. Next insert a light plug of cotton as indicated in the section on WHOLE LARVAE AND PUPAE above. Make certain that the skin(s) does not become entangled in the cotton or trapped between it and the walls of the shell vial. Place the label, insert the stopper and release the pressure as indicated in the section on WHOLE LARVAE AND PUPAE. Skins may also be transferred with a smooth-surfaced lifter but this takes great care and is not recommended for general use by inexperienced assistants.

After all the skins are processed a record should be made by a check mark on the back of the collection forms in the l (larval skins) or p (pupal skins) column on the line corresponding to the individual rearing number. Store and pack the vials as suggested in the next chapter.

INCOMPLETE REARINGS. All specimens that die during rearing in the laboratory whether in lot mass rearings, sublot mass rearings, or individual rearings should be preserved as carefully as all the other material. Also all skins from mass rearings (lot and sublot) should be preserved.

After the completion of lot and sublot mass rearings, process the plastic vials containing the skins and dead larvae and pupae in alcohol. Empty a vial into a sorting bowl (or smaller container) with fresh 80% ethanol. Transfer the skins (larval and pupal) into one shell vial filled with 80% ethanol and the whole larvae and pupae into another, following the procedure suggested above in the sections on WHOLE LARVAE AND PUPAE and on SKINS. Be sure to label everything and not to place more than a total of 20 specimens in any one vial. Record the material on the collection forms as indicated in the section on WHOLE LARVAE AND PUPAE.

All the material from incomplete individual rearings should be preserved in separate individual shell vials with 80% ethanol. A dead larva may be transferred directly into a shell vial but a moribund one should be killed in hot water following the procedure outlined on the section on WHOLE LARVAE AND PUPAE and then transferred to a shell vial after one passage through 80% ethanol without the hardening procedure.

EGGS. Samples of eggs should always be preserved from PROGENY REARINGS, EGG REARINGS and whenever possible from REARINGS FROM DRY MATERIAL (see preceding chapter). A sample of 5-10 eggs is usually sufficient but more should be preserved if the clutch is larger or if many eggs are collected in the field. The eggs of mosquitoes are still poorly known primarily because of failure to collect and preserve them for study. They show many valuable taxonomic characters and an effort should be made to collect and preserve them routinely.

Ten percent formalin (4% formaldehyde) should be used for killing and preserving eggs of the majority of species but 5% formalin may be advisable for the more delicate eggs of such forms as anophelines. Small shell vials with neoprene stoppers should be used for containers.

Egg rafts and hard-shelled aedine eggs are dropped directly into a shell vial filled with formalin. Use a moistened camel's hair brush to transfer the eggs from the water, paper toweling, cotton or other surface on which they are laid. In case of species of Mansonia which attach their eggs to various objects, all or part of this material around the eggs should be preserved. After the eggs have settled in the bottom of the shell vial insert a cotton plug (as with whole larvae and pupae) to just above the level of the eggs and place a label above the plug. Cap the vial and release the internal pressure in the vial with a needle inserted between the stopper and the inner wall of the vial.

Very delicate eggs with floats or processes (as in anophelines) should be killed and preserved by formalin fumes. First place the label facing to the outside against the side wall in the bottom of the shell vial and pack tightly some absorbent cotton in back of the label to a height of about 1 cm (about half an inch). Saturate the cotton with 5% formalin so that a very thin film remains on the surface. Next prepare a strip of filter paper or paper toweling narrow enough to slip into the shell vial and about 5 cm (2 inches) in length. Moisten this strip with 5% formalin and transfer very carefully with a moistened camel's hair brush one egg at a time to the middle part of the strip. Next fold over one end of the strip (without eggs) two or three times, the length of the folded part being less than the diameter of the shell vial, and slip the strip with the folded end first into the shell vial. Press the folded end against the cotton in the bottom and push the strip against the inner wall of the vial where moisture will hold it in place. Next cap the vial with the neoprene stopper in the usual way. DO NOT FILL THE VIAL with the formalin; the eggs will be killed and preserved by the fumes in the vial and the formalin on the strip picked up by capillarity from the cotton.

After the eggs have been thus processed a record should be made on the front of the collection forms in the section on SUBLOTS. Place a check mark in the E (eggs) column on the line corresponding to the sublot number; if only a lot number has been given use the line for sublot -1. Store and pack the material as suggested in the next chapter.

STORING, PACKING AND SHIPPING

STORING. All preserved adults and all supplies used for the preservation of adults must be stored in a dry pest-proof box or cabinet to protect them from molds, ants and particularly psocids. A simple wood cabinet with ventilation holes on top, an electric light bulb of low wattage (7.5 or 15 watts) as a drying source in the bottom and legs set in cans of oil serves very well. The temperature in the cabinet should be maintained as low as possible and should not exceed 40 C (104 F). If psocids are noted, "Dri-Die" or naphthalene flakes should be applied liberally in the cabinet. A small open cardboard container with thymol crystals should also be placed in the cabinet. Do not use insecticides such as DDT, BHC. If a drying cabinet is not available, plastic bags with silica gel should be used to store the material.

Gelatin capsules containing adults should be replaced in the original 100-capacity cardboard boxes. A few wisps of fluffed out cotton should be placed to keep the capsules from moving after the box is filled; do not use any undue pressure or the capsules will be crushed. The empty capsules should also be stored in the drying cabinet. The plastic pillboxes containing adults should

also be stored and packed in cardboard containers of a convenient size. Any space remaining in the container should be filled loosely with fluffed out cotton or crumpled paper to prevent movement of the pillboxes. The empty pillboxes should be kept closed and stored in the drying cabinet. The roll of household paper toweling and the squares of cut paper toweling to fit into the pillboxes should also be stored in the cabinet as well as the nylon netting.

The shell vials containing specimens, as well as other vials with ethanol, should be kept in a cool place, never in the drying cabinet. The shell vials should be packed neatly in cardboard containers of a convenient size or wrapped together in paper toweling in bundles of 20 to 50. Plastic vials containing alcohol should have the tops sealed with tape to prevent evaporation and be packed in small cardboard boxes.

PACKING AND SHIPPING. Material should be shipped as soon as possible for final processing and mounting. Do not let large quantities accumulate but send it in small parcels. Be sure to include the collection forms with the preserved specimens and indicate the letter code.

It is very important to pack the material very carefully or it may be completely ruined. Use the special shipping container consisting of a sturdy corrugated cardboard box lined with styrofoam sheets. All the material to be shipped must first be carefully packed in small cardboard boxes as indicated in the section above. Check every box for loose containers and fill in the unused space with cotton or crumpled paper so that nothing will move when the box is shaken but do not pack tightly. Next seal the individual boxes with tape. Place all the boxes in the shipping container and fill all the spaces between them tightly with crumpled paper so that nothing moves and the container is completely filled. Enough packing material should be placed on top of the boxes so that when the styrofoam lid is put on a slight pressure will be needed to keep it down level with the styrofoam sides. Now close the shipping container and seal the top with tape. The container should then be covered with wrapping paper.

If pinned specimens in Schmitt or other boxes are shipped, a corrugated board container lined with 2 inches of polyurethane foam should be used. Make certain that all the pins are in tightly in the box. Empty all the fumigation crystals or flakes from the box. If large "points" have been used in mounting specimens a pin on each side of every point should be used to prevent the point from swinging in transit.

If special shipping containers are not available use a sturdy corrugated board container with about 2 inches of tightly packed crumpled paper, excelsior or other packing material on all sides of the material which must first be packed inside a sealed smaller box. Never use a flimsy container and always fill with enough packing material so that the container will not be crushed.

All packages should be shipped by air, preferably by AIR PARCEL POST. If several are shipped at once it may be cheaper to use AIR FREIGHT but this may require special clearance by Customs and other government agencies and may actually cost more. All packages should be plainly marked "Preserved material for scientific study, no commercial value."

OUTLINE OF PROCEDURES

The following is an outline of the procedures set forth in the preceding pages of this paper for the handling of the mosquito material from the time of

its collection to the step of killing and preservation but does not include the details of killing and preservation. The purpose is to give an overall perspective of the procedures rather than to serve as the actual guide itself. Items in [square brackets] are optional but should be followed whenever possible.

Adults Collected

Killed upon capture:
- Killing tube (field)
- Place in plastic cup or vial with tissue paper (field)
- Preserve in pillbox (most mosquitoes) (lab)
- Preserve in alcohol (soft-bodied mosquitoes, gravid females not to be held for oviposition) (lab)

Females to be retained for progeny rearings:
- Place in oviposition vial (field)
- Transfer to oviposition cage (lab)
- Gravid females; sugar solution
- Non gravid females; sugar solution, daily blood meal

Progeny rearings
- 5-10 eggs preserved, formalin solution or fumes
- Transfer remaining eggs to proper type hatching vial
  - Follow by cycles of drying and flooding, if necessary
- Mass rearings in cup; daily feedings ground biscuit, chow or slurry
  - Preserve skins and 5-10 whole specimens of each instar
  - Preserve adults in pillbox
  - [Isolate 10 4th-instars for individual rearings
    - Preserve associated skins of each individual rearing
    - Preserve adults in gelatin capsules or pillboxes]

Pupae Collected

Not sorted to species:
- Isolate up to 15 into vials for individual rearings (preferably in field)
- 50 additional for mass rearing
  - Preserve skins, dead pupae and inviable adults in alcohol
  - Preserve adults in pillboxes
- Preserve remaining pupae

[Sorted to species:
- Isolate one half up to 10 of each species into vials for individual rearings
  - Preserve adults in gelatin capsules or pillboxes
- Mass rear one half and preserve one half of remainder (in alcohol)
  - Preserve adults in pillboxes]

Larvae Collected

Sort to species, each in a separate cup (in field if possible; complete in laboratory)
- Assign sublots, treat each as follows:
- Isolate 4th-instars into separate vials as individual rearings one half of total up to 10 (in field if possible, remainder in lab)
  - Rear to adult
  - Preserve all skins and inviable immatures and adults in alcohol
  - Preserve adults in gelatin capsules or pillboxes

Preserve 20 additional 4th-instars in alcohol
Mass rear remaining 4th-instars in cup(s)
    Follow special procedure for carnivorous forms
    [Preserve up to 10 whole pupae in alcohol]
    Rear remainder to adult; preserve all skins
    Preserve adults in pillboxes
Young larvae that cannot be sorted
    Mass rear noncarnivorous forms in original container to 4th-instar; then treat as above
    Rear suspected carnivorous forms in separate container(s)

Eggs Collected

Collected and transferred to field egg vial (field)
    Transfer to proper type of hatching vial (lab)
Collected in and together with dry material (field)
    Transfer to cup, flood, add vitamin C tablet (lab)
    Follow with several cycles of drying and flooding (lab)
Rear larvae through adult stage following procedure under "Larvae Collected"

GLOSSARY

Some of the common equipment and supplies used in mosquito surveys and rearings are listed below in alphabetical order together with explanations of some special terms or problems mentioned in this publication. The list is not meant to be exhaustive. Much useful information on equipment, methods of collecting and rearing and mosquito habitats will be found in other publications such as Belkin (1962: 67-82), Carpenter and LaCasse (1955: 3-5), Forattini (1962: 185-302, 593-642), Horsfall (1955), Howard, Dyar and Knab (1913: 106-185), King et al (1960: 11-17, 23-28), Matheson (1944: 80-86), Russell et al (1963: 283-361) and Trembley (1955) as well as in the specific references mentioned in the preceding text. It should be emphasized that existing methods should be adapted to local conditions and that new techniques should be tried. The mention of certain products, manufacturers and distributors in the glossary is not to be construed as endorsements of specific items; similar, equally satisfactory items may be available elsewhere.

AERIAL NET. Any kind of aerial net may be used but the bag should always be made of fine-meshed nylon marquisette (see NYLON NETTING). Very convenient is a small collapsible net with a short hollow aluminum handle into which a cane or stick may be inserted when needed.

ALTIMETER. Very convenient for field use is the compensating pocket altimeter manufactured by Lufft Instruments, distributed by Watrous & Co., Inc., 110 East 23rd St., N.Y., N.Y. 10010. The model with the meter scale is preferred.

AMBROID. This commercial liquid cement, manufactured by Ambroid Co., Inc., Boston, Massachusetts, is an excellent adhesive for attaching adult mosquitoes to paper points. The acetone solvent should be evaporated and replaced by amyl acetate before use.

AQUATIC NET. Various kinds of aquatic nets with circular, triangular or square frames may be used but the bag should always be of fine nylon marquisette (see NYLON NETTING).

ASPIRATOR (SUCTION TUBE). Convenient and practical aspirators can be made from 12.5 mm O.D. extruded acrylic plastic about 30 cm in length, provided with a hollow plug of 9 mm O.D. rubber or plastic tubing covered with a small piece of fine nylon netting (see) and a 60 cm length of 12.5 mm O.D. ordinary rubber tubing. If it is desired to taper the end of the aspirator the plastic tubing is heated over an electric hot plate (not an open flame), pulled out to the proper diameter and cut with a fine hacksaw blade.

BATTERY PIPETTE. Commercial battery hydrometers make excellent pipettes for collecting in axils, flower bracts and small treeholes. Discard the hydrometer element and cut off the external flange and the inner knobs on the rubber stopper. The hard rubber tubing should be substituted by a length of plastic tubing (extruded acrylic tubing 9 mm O.D.) that has been tapered as indicated under ASPIRATOR. The plastic tubing should be inserted through the rubber stopper so that its inner end is flush with the inner end of the rubber stopper inside the pipette.

CAGES (PLASTIC CUP CAGE, OVIPOSITION VIAL, PLASTIC VIAL CAGE). Any kind of container, plastic, glass or paper may be converted to a cage by attaching a screen top. We recommend the use of plastic vials for small cages and plastic cups for large cages.

CELLUCOTTON. Any kind of absorbent cotton processed into light weight sheets with smooth outer surfaces (not hard) may be used for protecting and preserving mosquitoes in pillboxes. If entomological cellucotton is not available surgical wadding or dental sponges serve the purpose very well. Small sheets of cellucotton should be cut very accurately to fit exactly inside the pillbox so that specimens cannot move into the adjoining layers. All cellucotton must be stored in a dry place free from molds, preferably in a plastic bag with silica gel.

COLLECTING BAG, EQUIPPED. A sturdy canvas field bag (surplus US Army or Marine Corps musette bag is very convenient) fully equipped with the following should always be carried on collecting trips: supply of collection forms, field book, maps, altimeter, thermometer, hand lens, pencils, grease pencils, label strips, paper towels and tissue, small scissors, forceps, camel's hair brushes, scalpel, pocket knife, machete, trowel, flashlight (torch), and a pair of light weight rubber or plastic boots.

COLLECTING CONTAINERS. A variety of containers, plastic, glass and cardboard have been used successfully for storage, transport and rearing of immature stages. Elaborate containers with vented stoppers (Russell et al 1963: 290) are not necessary. We recommend the use of PLASTIC CUPS (see) which are light weight, inexpensive and can be stacked when empty. See also PLASTIC SORTING CONTAINERS.

COLLECTION NUMBER (LOT). Number assigned to every collection and identifying all specimens from that collection; subdivided into sublots for different species (or individual females for progeny rearings) and individual identifying numbers for individual rearings.

COTTON (COTTON WOOL). The best grade of sterile absorbent cotton should be used. If tightly rolled, the cotton should always be fluffed out before use in shell vials, gelatin capsules or pillboxes. Cotton balls packed in plastic bags are convenient but still are too tight to use directly.

DIP NET AND BAG. A standard dip net frame, an aquarium net frame or a simple homemade wire frame of a diameter of about 15 cm (6 inches) may be used. The bag should be made of fine nylon netting (see) or bolting silk (No. 0) attached to a double muslin band. The depth of the bag should not exceed 10 cm and should be rounded not pointed.

DIPPER. Any standard mosquito dipper with a hollow handle to accommodate a stick or cane may be used. A white interior in the cup is helpful but not essential (nor is a long handle). A very practical dipper is an aluminum or stainless steel ladle of 8 oz (240 ml) capacity. Small pans of various kinds, bowls, cups and even spoons are also useful as dippers in some situations.

EMERGENCE VIAL. Plastic vial of 5 dram capacity containing pupa(e) for the emergence of the adult(s) of small to medium-sized species. For large species a 9 dram vial should be used. Keep stoppered with a solid polyethylene cap at all times.

ENTOMOLOGICAL FORCEPS. Featherweight spatulate-tip entomological forceps (similar to Ward's C310 or Turtox 110A435) should be used to transfer adult mosquitoes for storage and preservation. The adults should be grasped by the legs with the forceps.

GELATIN CAPSULE. Gelatin capsules make excellent containers for temporary storage of adult mosquitoes from individual rearings. Care must be taken, however, that the filled as well as the empty capsules never become wet and that they are stored in a dry and fairly cool place free from fungi and pests. Empty capsules are easily obtained in boxes of 100 from pharmaceutical distributors or drugstores throughout the world. Parke, Davis & Co. capsules no. 1 will accommodate most mosquitoes but it may be desirable to have at hand other sizes, from 000 (largest) to 5 (smallest). Under very humid conditions gelatin capsules may not be practical and should be substituted by small pillboxes.

GREASE PENCIL (CHINA AND GLASS, WAX). Glass and plastic marking pencil, the markings easily wiped off with cotton.

HOLDING VIAL. To make a holding vial for adults being hardened for a 24- or 48-hour period saturate a piece of absorbent cotton in fresh clean water and pack it very tightly in the bottom of a 5 dram plastic vial to a height of about 12 mm (1/2 in). For large species a 9 dram vial should be used. Keep stoppered with a solid polyethylene cap at all times. If any difficulty develops owing to moisture condensation in the vials substitute a fine-meshed nylon netting top for the solid cap.

INDIVIDUAL REARING. Individual reared in isolation so that the adult is associated with its pupal (pupal rearing) or its larval and pupal skins (larval rearing).

JERRY CAN. See PLASTIC WATER CAN.

KILLING TUBE, JAR. Chloroform is the preferred killing agent for adult mosquitoes but ethyl acetate may be used; cyanide is not recommended for general use. Killing tubes should be prepared with glass test tubes 25 mm x 200 mm (never plastic). A plug of sponge rubber or rubber bands cut into pieces are placed in the bottom of a test tube to a height of 20-25 mm (one inch or less). The rubber is saturated with chloroform and covered with a piece of crumpled ordinary paper (not absorbent) to a height of about 12-15 mm (about 1/2 inch) and topped with a circle of heavy white blotting paper. The tube is recharged before every trip by pouring in chloroform until the rubber swells. All excess chloroform and moisture must be wipped off thoroughly inside the tube. Two or 3 long strips of fine tissue paper should always be kept inside the tube to provide a grasping surface for dying mosquitoes. The strips should be replaced as soon as any evidence of moisture appears. The tube must be kept tightly stoppered with an ordinary cork. To reduce breakage of tubes wind adhesive tape around the outside of the bottom and rim. For ethyl acetate

tubes a layer of plaster of Paris is used in place of the rubber and crumpled and blotting paper. A killing jar is prepared by placing a ball of cotton saturated with chloroform or ethyl acetate in a sturdy wide-mouth glass jar, the top of the net containing the mosquitoes is introduced into the jar (not touching the cotton) and a solid screw top placed over it for a few minutes. It is not necessary to make a permanent killing jar for mosquitoes.

LACTOPHENOL. A mixture of 1 part phenol (absolute carbolic acid), 1 part absolute lactic acid, 2 parts glycerine and 1 part water has been widely used for killing and preserving whole larvae and pupae and preserving skins (Hopkins and Mattingly 1952: 30-31). Because of the difficulty of dehydrating material preserved in this viscous fluid satisfactory permanent mounts in euparal or balsam are seldom possible. Therefore the routine use of lactophenol is not recommended.

LARVAL FOOD. Individual and mass rearings of older instars collected in the field do not require food in addition to that brought in with the original water and concentrate. However, for mass progeny rearings and mass rearings of younger instars collected in the field it is essential to add a small amount of food regularly, preferably a very small amount twice a day. Finely ground laboratory chow, dog biscuit or similar dry animal food may be used. For surface feeders this is sprinkled on the water surface; for bottom feeders a slurry of food and water is first made and then placed in the container. Small amounts of yeast cake may also be used as specified in the text. We have found either dry or live pollen from various flowers and grasses effective food for hard-to-rear breeders in temporary pools and plant containers.

LARVAL REARING. See INDIVIDUAL REARING.

LIFTER. Various dental tools (probes, spatulas, etc.) may be used for transferring skins and whole larvae and pupae. The instruments should be perfectly smooth or damage to the specimens will occur. The use of lifters is not recommended for inexperienced personnel in the field; a medicine dropper or small pipette is safer.

LOT. Number assigned to a whole collection; see COLLECTION NUMBER.

MASS REARING. A general rearing without isolation of individuals. Three types are recognized, (1) lot mass rearing, rearing of a whole collection without separation into species, (2) sublot mass rearing, rearing of an individual species identified by a sublot number and (3) progeny mass rearing, rearing of the offspring of an individual female also identified by a sublot number.

MICROVIAL (PERFUME VIAL). Small glass vials, 7.5 mm x 50 mm O.D., 1 ml capacity (1/4 dram), provided with polyethylene stopper, commercially used as perfume sample vials, make excellent containers for the storage of small specimens. Because of the small capacity and narrow neck diameter some difficulty may be encountered with the larger larval and pupal skins. Therefore they are not recommended for routine use in the preservation of skins; use instead the larger SHELL VIALS (see). The microvials of the type described can be obtained from the Acme Vial and Glass Co., 4909 San Fernando Road West, Los Angeles, California 90039 (Acme long style patent lip vial, 1/4 dram, with AG 187 polyethylene stopper).

MOSQUITO PUMP. Homemade pumps are extremely useful for the collection of immature stages from crabholes, large treeholes and other confined habitats containing a considerable quantity of water. We have found the following two types, small and large, to be quite satisfactory. A rubber bulb, of the one-way, pressure or suction type (double acting), about 90 ml (3 oz) capacity, is satisfactory for either type of pump. Small type: The pump chamber

or reservoir is a rectangular milk bottle of 2 liter (2 quart) capacity. The rubber bulb is attached by a short length of plastic tubing to a 9 mm (3/8 in) copper tube fitting inserted with a sleeve of rubber tubing into a suitable hole bored into the milk bottle. A 1-2 meter (3-6 ft) length of rubber tube of either 12 or 25 mm (1/4 or 1/2 in) diameter is fitted onto a piece of aluminum tubing projecting through a rubber stopper which fits the mouth of the milk bottle. Large type: The reservoir is a plastic water can (Jerry can) of 9.5 liter (2.5 gals) capacity with a separate vent hole. The rubber bulb is attached to the vent hole of the water can through an identical copper tube fitting as above but without the rubber sleeve. The length of rubber tubing is fitted in the same manner as above to the pouring mouth of the water can. Either pump is operated by repeated rapid squeezing of the rubber bulb. Care must be taken not to overfill the reservoir or to get sand and debris into the rubber bulb. Smaller mosquito pumps, using mouth suction instead of a rubber bulb, may be useful for collecting in leaf axils.

NETS. See AERIAL, AQUATIC and DIP NET.

NYLON NETTING. Nylon marquisette is the preferred material for all netting (aerial and aquatic) and all screen tops. For nets a fine mesh (17 to mm, 42 to in) should be used; for screens on oviposition vials, plastic cup cages and so on, a coarser mesh (10 to mm, 25 to in) should be used to allow mosquitoes to feed through the screen.

OVIPOSITION VIAL, CAGE. To make an oviposition vial, saturate a piece of absorbent cotton in fresh clean water and pack it very tightly in the bottom of a 9 dram plastic vial to a height of about 12 mm (1/2 in). Cut a strip of paper toweling or filter paper about 2 cm (3/4 in) wide and 6.2 cm (2 1/2 in) long and fold 1.2 cm (1/2 in) at one end of strip. Insert the strip into the vial with the folded end resting on the wet cotton and the main part against the wall. Wet the strip with a drop of clean fresh water so that it clings against the wall. Remove all moisture from the inner walls of the vial with paper toweling and cap the vial with a solid polyethylene lid. Later, in the laboratory, the oviposition vial will be converted into a PLASTIC VIAL CAGE (see) by the addition of a netting top; the solid cap will be placed loosely over the netting top and the wad of cotton saturated with sugar solution.

PAPER TOWELING. For the storage and preservation of mosquitoes in pillboxes only soft light household paper towels in rolls (Scottowels or similar product) should be used. Cut the squares carefully to fit exactly into the pillboxes to prevent specimens from moving from the original layer. The paper toweling should be kept in a dry, fungus- and pest-free place. Coarse, tougher commercial grade paper hand towels should be used for oviposition strips and may be satisfactory for attachment discs for the immature stages of Mansonia.

PILLBOXES. Many different types of cardboard, plastic and metal pillboxes have been used for the storage and preservation of adult mosquitoes. Very satisfactory, uniform, inexpensive and readily available are small, clear, polystyrene boxes used for commercial packaging of small items. We have found 2 square boxes manufactured by Bradley Industries, Inc., 1650-58 N. Damen Ave., Chicago, Illinois 60647, to be very useful, Box no. 1 (3/4x3/4x 5/8 in) for individually reared adults and Box no. 3 (1 11/16x1 11/16x9/16 in) for mass-reared adults. See POLYSTYRENE PLASTIC for precautions to be observed with various solvents.

PIPETTE, MEDICINE DROPPER. A variety of pipettes have been used for the collection and transfer of immature stages. For the field we recommend

pipettes with a rubber bulb of 30 ml (1 oz) capacity and a 15 cm (6 in) length of 9 mm (3/8 in) extruded acrylic plastic tube. The end of the tube is tapered as indicated under Aspirator (see). Smaller pipettes can be made from 6 mm (1/4 in) glass tubing and a 10 ml rubber bulb. Very useful are polyethylene dropping pipettes whose tip can be cut to the right taper with scissors. Ordinary medicine droppers with the tip of the glass tube cut and fire polished to widen the diameter of the opening are recommended for the transfer of skins and whole larvae and pupae. See also BATTERY PIPETTE.

PLASTIC BAG. Plastic food bags (12 x 24 in) make convenient containers to store samples of soil and debris containing eggs.

PLASTIC CUP. The most practical containers for collection and mass rearings of immature stages have proved to be opaque white polystyrene cups with polyethylene caps used commercially for food packaging and as household refrigerator containers. They are inexpensive (cheaper than wax-lined paper containers), versatile, take up little space (stacking) and stand up well in the field. The 15 oz (450 ml), medium impact cup (HP 1215 MB) with polyethylene lid (HP 200), manufactured by Highland Printers & Plastic Molders, 1434 West Colorado Blvd., Pasadena, California 91105, has proved to be very satisfactory after several years of field and laboratory use. See POLYSTYRENE PLASTIC for precautions to be observed with various solvents. An ordinary pint cylindrical ice cream container fits into the flanged inner top of this plastic cup and can be used as an emergence cage by removing its bottom and screening its lid.

PLASTIC CUP CAGE. To make a plastic cup cage cut out the central part of the solid polyethylene cap and use the remaining rim to anchor a piece of nylon netting (see) over the cup. Make 2 slits at right angles to each other in the center of the netting wide enough to allow passage of an aspirator; stopper the opening with a solid plug of cotton. See POLYSTYRENE PLASTIC for precautions to be observed with various solvents.

PLASTIC PILLBOX. See PILLBOXES.

PLASTIC SORTING CONTAINERS. Various plastic containers (basins, bowls, buckets, pans) made of polyethylene or polypropylene are very useful for sorting and processing collections in the field or laboratory.

PLASTIC VIAL. Small, clear polystyrene vials with polyethylene caps are recommended as containers for individual rearings and with modification of the lid, as oviposition vials. The 5 dram (18.5 ml) and 9 dram (33.3 ml) vials, manufactured by Thornton Plastic Co., 745 Pacific Ave., Salt Lake City, Utah 84104, are very satisfactory. See POLYSTYRENE PLASTIC for precautions to be observed with various solvents.

PLASTIC VIAL CAGE. To make a plastic vial cage cut out the central part of the solid polyethylene cap and use the remaining rim to anchor a piece of NYLON NETTING (see) over the vial. See POLYSTYRENE PLASTIC for precautions to be observed with various solvents.

PLASTIC WATER CAN (JERRY CAN). A 20 liter (5 gallon) plastic container (polypropylene), designed for water storage, should always be carried on field trips filled with clean fresh water for washing container breeding sites and for sorting. It can be refilled in the field from springs or streams by filtering water through a dip net bag placed over the spout.

POLYSTYRENE PLASTIC. Paradichlorobenzene, chloroform, ethyl acetate and numerous organic solvents readily dissolve polystyrene and should never come in direct contact with plastic cups, plastic vials or plastic pillboxes.

POLYURETHANE. Soft spongy plastic upholstering material sold in sheets, recommended as packing insulation material for shipment of boxes of mounted adult mosquitoes.

PROCAINE CARTRIDGE. See SHELL VIAL.

PROGENY REARING (SERIES). Rearing from egg clutch laid by an individual female as a sublot mass rearing.

PUPAL REARING. See INDIVIDUAL REARING.

PUPATION VIAL. Plastic vial of 5 dram capacity containing an isolated larva being held for pupation. For large species use a 9 dram vial. Keep stoppered with a solid polyethylene cap at all times.

RACKS. Racks for holding plastic vials can be made simply by boring partial holes of appropriate diameter in slabs of styrofoam plastic (see).

REARING CONTAINERS. A variety of containers, plastic, glass and cardboard have been used successfully for rearing immature stages. We recommend the use of PLASTIC CUPS (see) for mass rearings and PLASTIC VIALS (see) for individual rearings.

SCREEN TOP. Screen tops for plastic cup or vial cages should be made from nylon netting (see). If mosquitoes are to be blooded through the screen use the coarser netting, otherwise the finer mesh. Cut out the center of the solid polyethylene cap and use the rim to attach the netting to the container.

SHELL VIAL, GLASS (PROCAINE TUBE OR CARTRIDGE). The most convenient containers for alcohol or formalin preserved specimens are small shell vials with neoprene stoppers. They are much more satisfactory than the procaine tubes or cartridges formerly widely used for this purpose. The filled shell vials should always be stored dry as there is practically no evaporation of alcohol through the neoprene stoppers. The shell vial itself can be made to order from ordinary no. 2 wall glass tubing of 8.75 mm diameter (8.55-8.95 mm) cut to a length of 64 mm by any commercial glass blowing firm. Stoppers can be obtained from the West Company, Phoenixville, Pennsylvania 19460, in a variety of sizes and compounds. For the vial diameter given above the following should be used: glass cartridge fitment, diaphragm no. 5, neoprene. The filled vial will contain, from bottom to top, the specimen(s), a loose cotton plug, the label and the stopper. It is very important, in capping the vial, to insert a dissecting needle between the neoprene stopper and the wall of the vial to release the gases under pressure; if this is not done the stopper may pop off later. A small air bubble should be present in the upper portion of the vial beneath the neoprene stopper but air bubbles should be excluded from the bottom portion of the vial beneath the cotton plug; air bubbles beneath the cotton plug may result in mechanical damage to the specimens when the vials are shaken, especially during shipping.

SIPHON. A length of rubber tubing may be used to siphon water from treeholes or other container habitats located above ground. Use mouth suction to start the siphon and collect water in a suitable container held below the level of the breeding site.

SORTING CONTAINERS. A variety of containers are used for sorting immature stages in the field (enamel pans, metal buckets and so on) but we recommend light weight PLASTIC SORTING CONTAINERS (see).

SQUEEZE BOTTLE (WASH). A plastic (polyethylene) bottle with attached tapered plastic tubing for dispensing liquids by squeezing the bottle is very handy for filling shell vials with alcohol. Never use the stream of alcohol directly on specimens.

STATIC ELECTRICITY. Difficulty is sometimes encountered with plastic

pillboxes or other containers becoming charged with static electricity with the annoying result that the specimens placed in the container are drawn to and cling to the walls. The static electricity can sometimes be discharged by touching the inside of the container with a metal object such as a pair of small scissors.

STERNO STOVE. A small simple collapsible field stove using cans of solidified alcohol as fuel; available in drugstores in most cities throughout the world; recommended for heating water for killing immature stages in the field.

STYROFOAM. Rigid, light weight expanded polystyrene plastic insulation material in sheets is recommended as packing insulation in shipping containers. Styrofoam can be used to make racks for plastic vials.

SUBLOT. Subdivision of a collection (lot) for individual species represented in that collection or for individual females in progeny rearings. See COLLECTION NUMBER.

SUCTION TUBE. See ASPIRATOR.

SURGICAL WADDING. See COTTON.

TISSUE PAPER. Soft absorbent tissue paper (facial, handkerchief, bathroom) should be used for strips in killing tubes or for protection of mass collections of adults in plastic cups or vials.

WATER CAN. See PLASTIC WATER CAN.

WET STRENGTH PAPER. Discs for oviposition and for the attachment of the immature stages of Mansonia should be cut from brown, wet strength crepe paper obtainable from paper houses in the larger cities. This tough paper is used for packaging and is treated with small amounts of a synthetic resin to reduce water absorption. If this paper is not available coarse commercial grade paper hand towels may be used.

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MOSQUITOES OF MIDDLE AMERICA

Code: _____ Date: _____
Number: _____ Collector: _____
Locality: _____ Nearest town: _____ Province: _____ Elevation: _____ m
_____ District: _____ Country: _____ Photo: _____

GENERAL ENVIRONMENT -1. Woody plants:height 0 - 2 - 8 - 15 - 30m; density 0 - 1 - 2 - 3 2. Herbs, grasses:height 0 - .5 - 1 - 2 - 5 - 8m; density 0 - 1 - 2 - 3 3. Epiphytes: 0 - 1 - 2 - 3 4. Edge or interior of vegetation, along road, dike, bank 5. Shore of sea, lake, stream mangrove, saltmarsh 6. Virgin vegetation, clearing, grazing, plantation, cultivation, domestic 7. Light:deep shade, partial shade, full sun
Annual rainfall: ca _____ cm Rainy season: J - F - M - A - M - J - J - A - S - O - N - D Vegetation type: _____

IMMATURE STAGES

Breeding site -1. Pond, lake 2. Ground pool: large, small 3. Animal tracks 4. Swamp interior, margin, marshy depression 5. Flooded forest 6. Seepage, spring 7. Well 8. Stream: margin, pool, blocked 9. Ditch, drain 10. Fountain, gutter 11. Crabhole:large, small 12. Rockhole:volcanic, coral stream margin, seaside 13. Artificial container: large, small: _____ 14. Treehole:large, small: _____ 15. Fallen tree: _____ 16. Bamboo: cut or broken, uncut internode 17. Animal container on ground: _____ 18. Fallen leaf, frond, spathe: _____ 19. Fallen fruit, nut, rind: _____ 20. Attached fruit: _____ 21. Leaf axil: epiphytic, terrestrial: _____ 22. Flower: bract, spathe: _____ 23. Pitcher: _____ 24. Trap: bamboo pot, _____ Height of site above ground _____ m

Water - 1. Permanent, semipermanent, temporary 2. Clear, turbid, colored: _____ 3. Stagnant, slow, moderate, strong current 4. Fresh, brackish, salty 5. Foul, slimy, fermenting

Vegetation in Breeding Site - 1. Abundant, scanty, none 2. Flotage, scum, algae 3. Grassy, herbaceous, woody, floating: _____, submerged: _____

Bottom - 1. Mud, sand, gravel, rock 2. Organic matter: plant _____ animal _____

ADULTS

1. Site: (specify exact situation) _____
2. Type: biting-landing, swarming, resting, sweeping, at light: _____, trap: _____
3. Height above ground _____ m
4. Host or bait: _____
5. Time of capture: _____

SUBLOTS

	Species	L	l	P	p	M	F	E
-1								
-2								
-3								
-4								
-5								
-6								
-7								
-8								
-9								

REMARKS

No _____ ✓ present 0 lost INDIVIDUAL REARINGS + dead, preserved in alcohol

Sub	Species	l	p	M	F	Sub	Species	l	p	M	F	Sub	Species	l	p	M	F	Sub	Species	l	p	M	F
-100						-21						-47						-73					
-101						-22						-48						-74					
-102						-23						-49						-75					
-103						-24						-50						-76					
-104						-25						-51						-77					
-105						-26						-52						-78					
-106						-27						-53						-79					
-107						-28						-54						-80					
-108						-29						-55						-81					
-109						-30						-56						-82					
-110						-31						-57						-83					
-111						-32						-58						-84					
-112						-33						-59						-85					
-113						-34						-60						-86					
-114						-35						-61						-87					
-10						-36						-62						-88					
-11						-37						-63						-89					
-12						-38						-64						-90					
-13						-39						-65						-91					
-14						-40						-66						-92					
-15						-41						-67						-93					
-16						-42						-68						-94					
-17						-43						-69						-95					
-18						-44						-70						-96					
-19						-45						-71						-97					
-20						-46						-72						-98					

Fig. 1. Collection Form

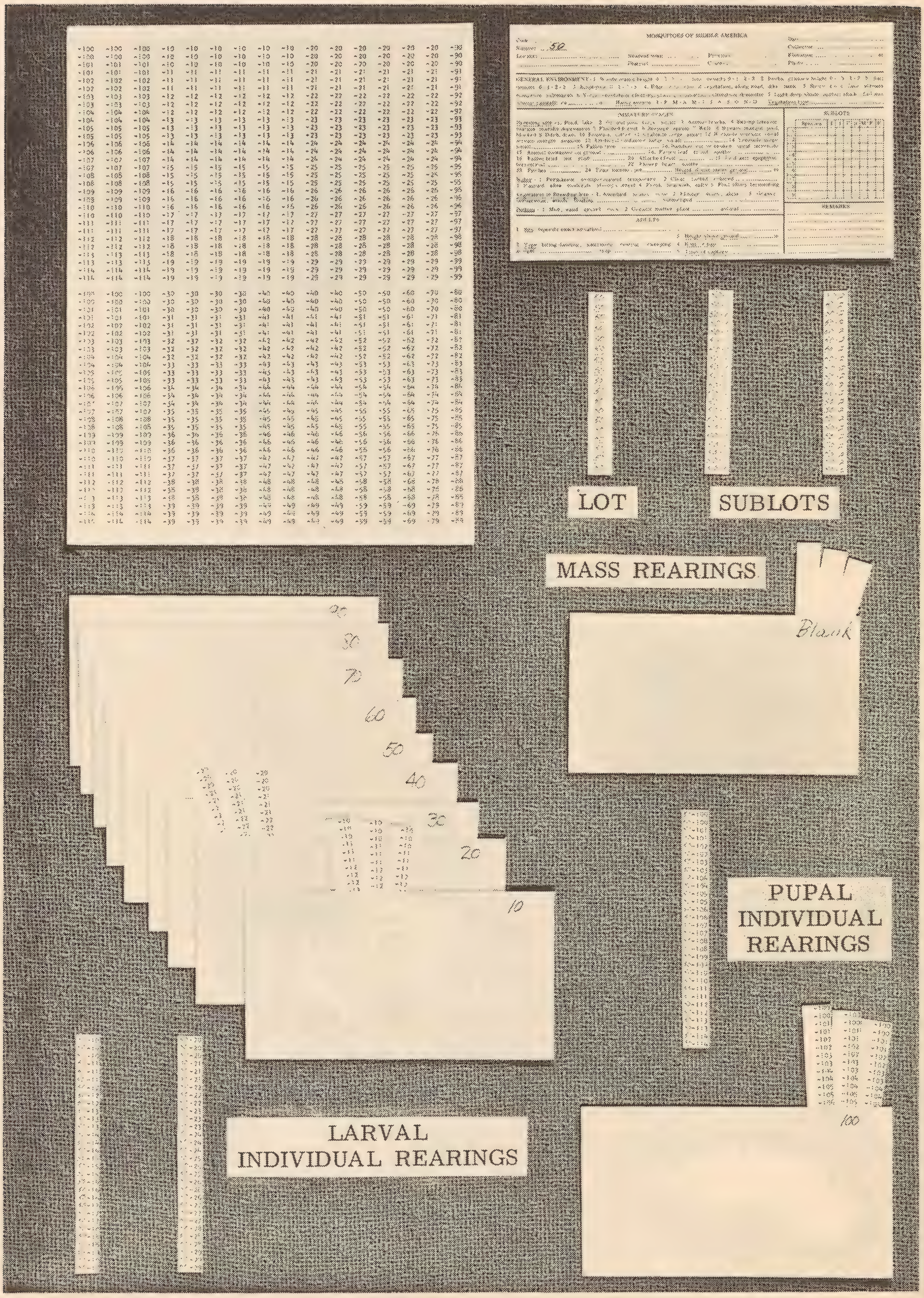

Fig. 2. Collection and Rearing Labels